MATING TYPES AND THEIR INTERACTIONS IN THE CILIATE INFUSORIA[1]

INTRODUCTION

DR. H. S. JENNINGS

JOHNS HOPKINS UNIVERSITY

THIS program deals with phenomena in Protozoa that are related to, or analogous to, the diversity of sexes.   But a wider view than that of sex diversity is here necessary, as you will see.   We are to deal with more general phenomena, of which sex diversity is but one example.   We are to deal with the differentiation of organisms into diverse classes, two or more than two, such that members of the same class do not unite for reproduction, while members of different classes *do* so unite.   We are calling these diverse classes *mating types;* from this point of view the two sexes in higher organisms are mating types.

The program deals with these phenomena in the ciliate infusoria; and particularly but not exclusively in that familiar acquaintance of all of us, and of many that are not zoologists—Paramecium, so generally employed as a type for Protozoa.

Before we begin we wish to try to show by demonstration on the screen, or by photography, or both, the remarkable phenomena in which the difference of type manifests itself, in order that you may realize that we are not talking of doubtful or obscure things, but of striking reactions, things

[1] Presented at the joint symposium of the American Society of Zoologists and the Genetics Society of America in conjunction with the American Association for the Advancement of Science at Richmond, Virginia, December 30, 1938.

that "leap to the eye." Individuals that belong to the same mating type—for example, individuals of a single clone, all derived from the same parent by fission—do not conjugate together. But when individuals of two diverse mating types are mixed, they immediately agglutinate— gather together in large clumps in which the individuals are literally stuck together. From these clots the individuals later emerge as pairs, each pair consisting of one individual of each of the two mating types.

We are going first to venture to try to show these things in the living organisms projected on the screen. The animals that we show are *Paramecium bursaria,* the green Paramecium.

[Here followed demonstrations on the screen, as follows: (1) Collection of living individuals all belonging to the same mating type: they do not clump together, but remain swimming about singly. (2) A mixture of individuals belonging to two mating types, three to five minutes after the mixture was made. The individuals have clumped together into large masses. (3) A collection of individuals belonging to a single mating type was projected on the screen: to this was added, in full sight of the audience, individuals of another mating type: the two types clumped together immediately into dense aggregations. (4) A later stage of the aggregations, after five or six hours: the clumps were partly disintegrated into small masses, chains of individuals, pairs and single individuals. (5) The condition about 24 hours after the mixture was made: almost all the individuals united in pairs.]

Photographs of four successive stages in the mating behavior are shown in Fig. 1.

For the ciliate Protozoa, observations that may now be interpreted as indicating a differentiation into diverse mating types were first made in the monumental work of Maupas (1889), published just fifty years ago. We shall be pleased if this program may be considered a jubilee celebration of the great work of Maupas, which has so profoundly influenced all work on the biology of Protozoa.

In four species of cilates—*Leucophrys patula, Onycho-dromus grandis, Stylonychia pustulata* and *Loxophyllum fasciola*—Maupas observed that there is no conjugation
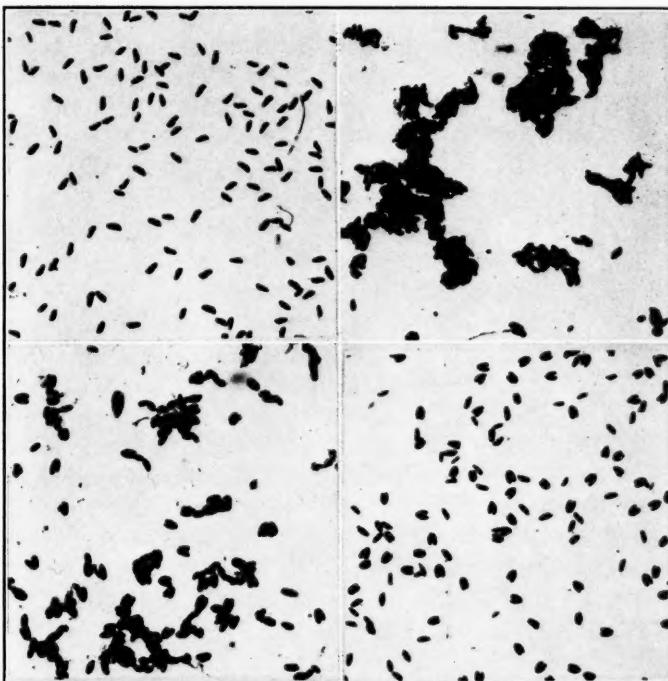

FIG. 1. Photographs of the mating behavior in *Paramecium bursaria*. Upper left, individuals of a single type (C), scattered and swimming singly. Upper right, mixture of individuals of two diverse mating types (P and Q), six minutes after the two were mixed. The individuals clumped into large masses. Lower left, mixture of two types (P and Q) about five hours later. The large masses have disintegrated into small masses, chains and pairs. Lower right, mixture of two types (P and Q) 24 hours after the mixture was made. Most of the individuals in conjugated pairs.

among the descendants by fission of a single individual—among the members of what we now call a *clone*. But if two clones of diverse origin are mixed, conjugation between their members, he observed, commonly occurs.

Maupas believed that this was a matter of closeness of relationship; closely related individuals, he held, do not mate together, while more distantly related ones may do so. As we shall show, this was not an adequate or accurate statement of the situation.

These observations of Maupas appear not to have been taken up and developed for the ciliate infusoria up to the time of the work which we are presenting to-day. I did myself attempt earlier to examine into the matter, but was so unfortunate as to select for intensive study *Paramecium aurelia.* In this species, as it turns out, conditions are exceptional and very different from those described by Maupas. Conjugation frequently occurs between members of the same clone, between individuals showing the closest possible relationship. The situation here could not be understood until knowledge had advanced much farther, in a number of different directions.

This led to a turning aside from the study of these matters. It was only when, in the course of our comprehensive study of the genetics of *Paramecium aurelia,* Sonneborn undertook and carried out a detailed study of the genetic consequences of endomixis, that he discovered the key to the situation; and this opened up the entire subject.

We plan to present to you this morning the main concrete facts and relations that have thus far been discovered in the free-living ciliate infusoria. In that presentation you will observe two general relations that are of interest and that may be stated in general terms at the beginning.

The first is that the conditions found are extremely diverse in different species, and even in different races of the same species.

The second is related to the first; it may be expressed as follows: In any species or race the conditions fall into a system that is clearly marked and unmistakable, a system comparable to that of sex diversity and sex determination in higher organisms. Yet to many features of this system there are exceptions; there occur rare exceptional conditions that do not conform to the system. In a given spe-

cies or race most of the individuals operate in accordance
with the system, but occasional individuals do not.   Such
exceptional conditions are of course not unknown in the sex
determination system of higher organisms; in the Protozoa
as in higher organisms they are of much interest.   They
possibly indicate that the systems under study are in a
state of evolutionary flux.

One word further as to a wider outlook in these studies.
In the unicellular organisms we have the last refuge of
"the inheritance of acquired characters."   Particular en-
vironmental conditions—unusual temperatures, chemicals
or the like—produce definite changed characteristics in the
organisms, and these "acquired characters" are inherited
for many generations of vegetative reproduction; they
have been called by Jollos "Dauermodifikationen."   These
"long-lasting modifications" are commonly, though not
always, changed or lost at conjugation or at endomixis.
Just what is the nature and seat of these inherited environ-
mental characters?   To discover this, we must first work
out in full and in detail the normal genetics of these organ-
isms; that is what we are now attempting.   Then by cross-
ing modified and unmodified individuals we may hope to
discover the secret of the inheritance of these environmen-
tal modifications.   An understanding of the relation of en-
vironmental changes to inherited changes is one of the
greatest needs of genetic science.   Perhaps the most direct
approach to this problem lies in work on the genetics of
these unicellular organisms.

For the presentation of these matters, we have prepared
a program of two longer papers, dealing with a number of
diverse aspects of the subject; then three shorter papers
dealing with single aspects of the matter in particular
organisms.

PARAMECIUM AURELIA: MATING TYPES AND GROUPS; LETHAL INTERACTIONS; DETERMINATION AND INHERITANCE[1,2]

DR. T. M. SONNEBORN

DEPARTMENT OF ZOOLOGY, JOHNS HOPKINS UNIVERSITY

IN *Paramecium aurelia,* the progeny of a single individual do not as a rule conjugate with each other, so long as nuclear reorganizations have not occurred; but when the progeny of certain different individuals are brought together under the proper conditions, they give at once a striking agglutinative sex reaction. Clumping of the individuals is followed by pairing and conjugation. The group of vegetative progeny of one individual, among which conjugation does not ordinarily occur, is called a *caryonide.* Two caryonides that do not conjugate by themselves, but do conjugate when brought together are said to be of diverse *mating types.* Sonneborn (1937) showed that in one stock (S) of *P. aurelia,* all the caryonides were of either one or the other of two mating types, designated I and II.

To ascertain the mating type of a caryonide it is required to mix some of its members with standard cultures of each of the mating types; with one and only one of these it will conjugate: *e.g.,* if it is type I it will conjugate in the mixture with type II, not in the mixture with type I. The mating type of a caryonide, therefore, is the opposite of the type with which it conjugates, the same as the one with which it fails to conjugate.

The composition of the species *P. aurelia* in relation to mating types was ascertained by examining the descendants of representatives of the species isolated from some 30 different ponds and streams in the eastern United States

and California. The progeny of a single wild individual are referred to as a stock. The first problem was to ascertain whether mating types occur in each stock. This was done by obtaining in each stock many caryonide cultures descended from different exconjugant or otherwise reorganized individuals, and mixing samples of each caryonide with a sample of each of the other caryonides. Observations were then made as to which mixtures resulted in conjugation and which did not. The results fell into two diverse systems.

In all the stocks except six, the system of results was like that shown in Table 1 for stock Q. Here, + signifies that the sex reaction and conjugation occurred in mixtures

TABLE 1

Paramecium aurelia, stock Q. Results of mixing different caryonides. + = conjugation ; − = no conjugation. In each square is given the result of mixing the caryonides on the corresponding row and column.

CARYONIDES

	2b1	3b1	4b1	5b1	7b2	13b1	16a2	30a2	1b2	2b2	3b2	4b2	5b2	7b1	13a2	19b1	21b2
2b1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
3b1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
4b1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
5b1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
7b2	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
13b1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
16a2	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
30a2	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
1b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
2b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
3b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
4b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
5b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
7b1	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
13a2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
19b1	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
21b2	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−

CARYONIDES

STOCK Q

of the two caryonides represented on the corresponding
row and file and — signifies the sex reaction and conju-
gation did not occur.   It appears that the caryonides are
of two diverse types; mixture of any caryonide of the one
type with any caryonide of the other type results in the
sex reaction and conjugation; but mixture of two caryon-
ides of the one type or of two caryonides of the other type
does not result in the sex reaction or conjugation.   These
two kinds of caryonides are thus of two different mating
types, and in such a stock every caryonide will react
sexually and conjugate with either one or the other of the
two mating types; ordinarily no caryonide will react with
both types.   This general relation is based on the study—
in some stocks—of many hundreds of caryonides.

The remaining six stocks show a different system, illus-
trated by stock B in Table 2.   In these stocks the sex
reaction and conjugation never occur in mixtures of dif-
ferent caryonides; not merely the 20 caryonides shown in
the table, but literally hundreds and thousands of caryon-
ides have been examined with the same result.   The
explanation of this system of results appears at once when
caryonides of such stocks are mixed with the diverse mat-
ing types found in certain other stocks.   In the case of
stock B, for example, all its caryonides give the sex reac-
tion and conjugate when mixed with one of the two mating
types found in stock S, type II, but not when mixed with
the other one, type I.   Hence, *all* caryonides of stock B
are of mating type I; they are identical in behavior with
type I of stock S: they conjugate with type II, but not with
type I.   On analysis, it is found that all 6 of the stocks
that fail to conjugate when diverse caryonides *within* the
stocks are mixed, do conjugate when mixed with one, but
not when mixed with the other, mating type in certain
stocks.

In sum, of the 36 stocks investigated, 30 consist of two
interbreeding mating types and the remaining stocks con-
sist of but a single mating type each.   In *P. aurelia,* mating
types appear to be of universal occurrence: each caryonide

TABLE 2

Paramecium aurelia, stock B. Results of mixing different caryonides with each other and with mating types I and II of stock S.   + = conjugation ; – = no conjugation.   In each square is given the result of mixing the caryonides on the corresponding row and column.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	I	II
	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Stock B	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
CARYONIDES	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Stock S Types	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		+
	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–

Stock B

in every stock belongs to some definite mating type.   There are no non-conjugating stocks or caryonides.

Are there but two mating types among all these stocks? This question was tested directly by mixing the types found in each of 17 of the stocks with the types found in each of the other 16 stocks.   The results of these 496 different mixtures are shown in Table 3.   It appears that while some stocks contain the same two types, others contain two different types.   Altogether, however, only 6 different types exist.   In one group of stocks (B, F, J, etc.) the two types, I and II, are found; mating type I from any of these stocks reacts and conjugates with mating type II

TABLE 3

Results of mixing in all possible combinations of two the mating types found in 17 different stocks of *Paramecium aurelia*. The capital letters give the names of the stocks, the Roman numerals designate the mating types. + = conjugation ; − = no conjugation. In each square is given the results of mixing the stocks and types on the corresponding row and column.

	B I	F I	J I	R I	S I	F II	J II	R II	S II	A III	C III	D III	E III	G III	H III	K III	L III	A IV	C IV	D IV	E IV	G IV	H IV	K IV	L IV	W IV	M V	Q V	Y V	M VI	Q VI	Y VI
B I	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F I	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
J I	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R I	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S I	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
F II	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
J II	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R II	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S II	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
C III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
D III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
E III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
G III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
H III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
K III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
L III	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
A IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
C IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
G IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
K IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
W IV	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
M V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Q V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Y V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
M VI	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
Q VI	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
Y VI	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−

from any stock; but neither of these types will conjugate with the same type from the same or a different stock. Nor will either of these mating types conjugate with any of the other stocks. A second group of stocks (A, C, D, etc.) shows an independent but similar mating system: the two mating types in these stocks are called III and IV, since they are different from (*i.e.*, do not mate with) the types I and II. Mating type III, from any of this second group of stocks, gives the sex reaction and conjugates when mixed with type IV from any of the stocks, but neither of these types will conjugate with the same type from the same or a different stock. Finally, there is a small third group of

stocks (M, Q and Y) with two mating types that do not conjugate with any of the types I, II, III or IV and so require different designations. These two types, V and VI, show again the same system of interbreeding shown by the first two pairs of types: V from any stock mates with VI from any stock, but no two V's or two VI's will interbreed.

The system of mating types shown by these 17 stocks is illustrated in condensed form in Table 4. The species

TABLE 4

The system of mating types in *Paramecium aurelia*. The six mating types fall into three groups of two types each, with conjugation only between the two types of the same group, never between different groups.
+ = conjugation ; − = no conjugation.

			Group or variety					
			1		2		3	
		Mating type	I	II	III	IV	V	VI
Group	1	I	−	+	−	−	−	−
		II	+	−	−	−	−	−
or	2	III	−	−	−	+	−	−
		IV	−	−	+	−	−	−
Variety	3	V	−	−	−	−	−	+
		VI	−	−	−	−	+	−

consists of 3 diverse groups of stocks. Each group contains two mating types that interbreed with each other: Group 1 with its mating types I and II; Group 2 with its mating types III and IV; Group 3 with its mating types V and VI. But there is no conjugation between the different groups. However, many stocks exist within each group and most of these contain both the mating types characteristic of the group, a few containing only one of them. This system shows that to test a new stock it is necessary merely to mix its caryonides with each of the six mating types: any one caryonide will react with only one of the six types and all other caryonides of the stock will react with either the same type or with the other type found in the same group. This method of analysis was applied to the remaining 19 stocks. Six of them were thus found to belong to Group 1, 11 of them to Group 2 and 2 of them to Group 3.

The division of the species *P. aurelia* into non-interbreeding groups of mating types agrees with the situation previously found in *P. bursaria* by Jennings (1938).

There is some slight evidence that at least two of the three diverse pairs of mating types from different groups of stocks of *P. aurelia* may be derived from one original pair of types. Although no conjugation occurs between the different groups, mixture of type II from Group 1 with type V from Group 3 does result in a very weak sex reaction: pairs form repeatedly for a few moments at a time, then break apart. Thus, types II and VI are similar in that both react with type V; and types I and V are similar in that both react with type II. This suggests that I and V are diverse modifications of one original type, while II and VI are diverse modifications of another original type. No such relation has been found between any other two types from different groups.

The existence of three non-interbreeding groups of stocks raises the question of whether each group is a distinct species. If the groups differed consistently in any morphological respects, the answer would be clear; but such differences have not been observed. All conform to the description of *P. aurelia*. Even in the absence of such differences, the thoroughgoing sexual isolation of the three groups is held by some geneticists to be sufficient ground for considering them as distinct species. Such a stand, however, affords serious practical difficulties for investigators not in a position to make the experiments necessary for identification. I therefore propose to consider the three diverse groups of stocks as three varieties of *P. aurelia*, varieties 1, 2 and 3. As will appear, the three varieties differ in a number of physiological respects.

The most striking differences among them are in the conditions required for conjugation: the necessary conditions of temperature and time of day differ for the three varieties.

Variety 1, with mating types I and II, gives the sex reaction and conjugates at any hour of the day and at any temperature within the range examined (9°–32° C.).

Variety 2, with mating types III and IV, will give the sex
reaction and begin to conjugate only at temperatures
below 25°, best below 20°, and only between 6 P.M. and
10 A.M., best between 1 A.M. and 5 A.M.   Variety 3, with
mating types V and VI, will give the sex reaction and
conjugate only below 28°, best below 24° C., and only
between 1 A.M. and 1 P.M., best between 4 A.M. and 11 A.M.
In variety 3, it has been shown that this diurnal periodicity
is a consequence of the daily alternation of light and dark-
ness.   After 5 days of continuous darkness, mating reac-
tions occur equally well at any hour of the day or night;
after 5 days of continuous illumination, the mating reaction
will not occur at any hour of the day or night.   That the
effective action here is not a stimulation by darkness, but
an inhibition of the sex reaction by light is evident from
further experiments.   When cultures are put into dark-
ness at different hours, they all begin to react at the same
time; hence darkness does not stimulate reactivity.   When
put into darkness at the same time and exposed to light at
successive hours next day, the later they are first exposed
to light the later they continue to react; hence light sup-
presses reactivity.

Lethal Interactions Between Diverse Stocks

In addition to the varietal differences in conditions re-
quired for the sex reaction and conjugation there are also
differences with respect to the effects of lethal substances
produced by some of the stocks.

When the mating types of different stocks were mixed
together by twos in all possible combinations, as earlier
described, it was observed that in many of the mixtures
there appeared animals abnormal in form, structure and
behavior, and ultimately many corpses, so that in these
mixtures many of the animals die.   The mixtures that
showed such phenomena among 26 of the stocks are indi-
cated in Table 5 by L; those that did not, by O.   As is
evident, only those mixtures which included stocks E, G
or H (all three belonging to variety 2) gave these peculiar
results.   It was further discovered that the same conse-

TABLE 5

P. aurelia. Lethal interactions in mixtures of different stocks. L = lethal inter-
action in mixture of stocks on corresponding row and file. O = no lethal inter-
action. In the lethal mixtures involving stocks E and G, the animals
spin around the longitudinal axis during the days prior to death. In
the lethal mixtures involving stock H the animals become slug-
gish and vacuolated in the hours prior to death. In the
mixtures of stocks E and G with stock H, the H lethal
effect appears, not the E or G effect.

Variety	Stock	B	F	J	N	O	P	R	S	T	Z	A	C	D	E	G	H	I	K	L	U	V	W	X	M	Q	Y
1	B	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	F	O	O	O	O	O	O	O	O	O		O	O	O	L	L	L	O	O	O	O	O	O	O			
	J	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	N	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	O	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	P	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	R	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	S	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	T	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	Z	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
2	A	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	C	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O
	D	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	E	L	L	L	L	L	L	L	L	L	L	L	L	L	O	O	L	L			L	L	L	L	L	L	L
	G	L	L	L	L	L	L	L	L	L	L	L	L	L	O	O	L	L	L	L	L	L	L	L	L	L	L
	H	L	L	L	L	L	L	L	L	L	L	L	L	L	O	L	L	L	O	L	O	L	O	L	L	L	L
	I	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	K	O	O	O	O	O	O	O	O	O	O	O	O	O		L	O	O	O	O	O	O	O	O		O	O
	L	O	O	O	O	O	O	O	O	O	O	O	O	O		L	L	O	O	O	O	O	O	O	O	O	O
	U	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O
	V	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	W	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	X	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
3	M	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	Q	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O
	Y	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O

quences developed if the other stocks were put in fluid in
which stocks E, G or H had lived. These effects were
shown not to be due to any contaminant in the fluid, for
thorough washing of the animals of stocks E, G and H was
followed by the production of lethal fluid again. More-
over, the lethal power of such fluid quickly wore off in the
absence of animals of the three stocks involved: its activity
was lost in 3 days at 10° C., in 1 day at 30° C., in 1 minute
at 50° C. Obviously, the animals themselves of stocks E,
G and H make the fluid in which they live lethal to other
stocks. The lethal substances require different times to

produce their first visible effects: 2 to 4 hours in the case
of the one produced by stocks E and G, 12 to 18 hours in
the case of the one produced by stock H; each produces
characteristic symptoms; to each there are hereditary
differences in reaction and in susceptibility; each is active
in very great dilutions.

The E and G lethal substances appear to be identical
in their effects. They make animals stop feeding, develop
many crystals in the cytoplasm, and spin vigorously on
their longitudinal axes. Within a few days, all die, even
if they are washed free of the lethal fluid and carefully
cultured.

The H lethal substance produces very different effects.
It makes animals of susceptible races stop feeding, become
sluggish and badly vacuolated and die within 36 hours.

The two kinds of lethal substance not only produce
diverse effects on the same race; but each lethal substance
produces somewhat diverse effects on the different races
and varieties of the same species. The E and G lethal
substance acts quickly and violently on varieties 1 and 3
of *P. aurelia,* but very slowly and slightly on variety 2.
It is easy to discover within 24 hours whether or not a new
and unknown stock belongs to variety 2 by this simple test
without making the elaborate preparations and tests to dis-
cover its mating types and conditions for conjugations.
The H lethal substance also acts quickly and strongly on
varieties 1 and 3 and also on a few races of variety 2; but
most races of variety 2 show varying degrees of lesser
susceptibility, and some are completely immune. If an
unknown new race shows little or no effect of the H lethal
substance—and this is discoverable in 24 hours—it is
certain that it belongs to variety 2. Thus, variety 2 is
characterized by including races that can produce these
lethal substances and usually by a high resistance or com-
plete immunity to them; while varieties 1 and 3 apparently
do not produce lethal substances and are highly susceptible
to them. Similar differences in reaction and sensitivity
to these two lethal substances have been found among other

species of Paramecium and, in general, resemble similar interactions known among the Fungi (see review by Porter and Carter, 1938).

These lethal interactions throw light on certain problems of ecology and evolution. They show that certain stocks of the same species and certain combinations of species could not coexist in nature. Varieties 1 and 3 of *P. aurelia* are thus at a great disadvantage in the struggle for existence, as compared with variety 2; and this is perhaps an explanation of the fact that the majority of natural collections of *P. aurelia* consist exclusively of variety 2.

Determination and Inheritance of Mating Type

From the foregoing, it is evident that discovery of mating types in *P. aurelia* has led to a radical change in knowledge of the composition of the species and of the interactions between its component races and varieties. It is also leading to equally radical changes in knowledge of heredity in this species. The genetic analysis has been pursued farthest in variety 1, so that most of the following account refers to this variety only. On certain points, results obtained with variety 3 are given for comparison; but variety 2 is left entirely out of consideration, for it has not yet been sufficiently investigated from a genetic point of view.

The inheritance of mating type follows different rules and shows different features in genetically diverse material. It is therefore necessary to take up separately: (1) stocks that contain two mating types, (2) stocks that contain only one mating type, and (3) hybrids between these two kinds of stocks and their descendants.

(1) *Stocks that contain two mating types.* In stocks containing two mating types, the types are ordinarily strictly inherited without change within the caryonide, *i.e.*, during vegetative reproduction. To this general rule exceptions of great interest occur rarely. These have been investigated by Kimball; an account of his discoveries will be set forth later. I turn now to the inheritance of mating types at nuclear reorganization.

There are at least two kinds of normal nuclear reorgani-
zation processes in *P. aurelia*. In one of them, conjuga-
tion, two individuals of different mating type unite, fer-
tilize each other and separate. From the fertilization
nucleus each exconjugant then develops a new nuclear
apparatus which is, therefore, biparental in origin. An-
other type of nuclear reorganization occurs periodically
within single individuals, without the introduction of
nuclear material from another individual. In this type
of reorganization, according to Woodruff and Erdmann
(1914), neither gamete nuclei nor syngamy occur; but
Diller (1936) holds that they do. The former call this
type of reorganization endomixis, the latter autogamy. To
distinguish this type of reorganization from conjugation,
without prejudice as to the exact processes involved, it will
be referred to as uniparental nuclear reorganization. Per-
haps it occurs sometimes in the form of endomixis, some-
times in the form of autogamy. Genetic evidence on this
will be presented towards the end of this paper.

At conjugation, the mating types show the same features
of inheritance in both variety 1 (types I and II) and variety
3 (types V and VI). Among a set of exconjugant indi-
viduals some give rise to progeny all of one mating type,
some give rise to progeny all of the other mating type and
some give rise to two diverse lines of descent, one of one
type and the other of the other type. In those exconjugant
individuals that give rise to both mating types, these
segregate as a rule at the first exconjugant fission: of the
two products of the first fission, one and all its vegetative
descendants are of one mating type, while the other and
all its vegetative descendants are of the other mating type.

At uniparental nuclear reorganization, Kimball (1937)
found the same set of relations in stock S of variety 1: some
reorganized individuals yield progeny of one type, some
the other type, and some both types. As at conjugation,
when the latter occurs, the types segregate at the first
fission after the new nuclear apparatus is formed. I have
confirmed Kimball's results in other stocks of variety 1
and also in variety 3.

Thus, in these stocks, the mating types arise in essentially the same way after conjugation and after uniparental nuclear reorganization. Further similarities in the rules and ratios of inheritance will appear below. Apparently the introduction of nuclear material from another individual does not influence the phenomena with which we here deal. The determination of mating type appears to be governed by some process common to both conjugation and uniparental nuclear reorganization. The clue to the process involved is given by those reorganized individuals from which two lines of descent differing in mating type arise at the first fission. At the same fission, each of the two resulting individuals receives one of the two new macronuclei normally formed in each reorganized individual. Are the mating types determined by the macronuclei? Critical evidence on this question is afforded by the exceptional relations found in certain stocks.

In stock R of variety 1, the mating types I and II frequently segregate at the *second* fission of the exconjugant, instead of at the first one. If the macronuclei determine the mating types, then there should be more than two new macronuclei formed in those reorganized individuals which yield segregation of mating type at the second fission. In agreement with this, cytological investigation showed that in stock R about 20 per cent. of the reorganized individuals contained more than two new macronuclei: in most cases only three or four, but in a few cases up to ten. In this stock, therefore, it frequently requires two, and very rarely three, fissions before the new macronuclei formed at nuclear reorganization are segregated to different individuals. The frequency with which mating type segregated at the second fission was found to agree with the expected frequency if mating type were dependent upon the macronuclei. This exceptional evidence, together with the normal case, the evidence set forth below in relation to the effect of temperature on the mating type ratios, and other lines of evidence that time does not permit me to review here, make it perfectly clear that the mating types are determined by the macronuclei.

This then is the basis of the term "caryonide." A caryonide is a group of individuals having macronuclei descended by division from a single original macronucleus. Such a group of individuals are ordinarily alike in mating type. Each reorganized individual usually gives rise to two caryonides; in some stocks, occasionally more. The caryonides produced by a single reorganized individual are sometimes alike, sometimes different in mating type. Each caryonide comes to an end at the next nuclear reorganization, when the old macronuclei disintegrate and new ones are formed.

The relative frequency with which caryonides of each mating type appear after nuclear reorganization (both conjugation and uniparental reorganization) differs in different experiments. Table 6 summarizes the results of many

TABLE 6

P. aurelia. Stocks with two mating types.
Inheritance ratios at conjugation and uniparental nuclear reorganization.

Variety 1							
	Number of caryonides						
Mating Type I	128	111	391	103	212	15	83
Mating Type II	63	91	394	133	426	38	279
Ratio II/I	0.5	0.8	1.0	1.3	2.0	2.5	3.4

Variety 3							
	Number of caryonides						
Mating Type V	116	166	103	83	77	25	40
Mating Type VI	108	202	143	139	157	62	138
Ratio VI/V	0.9	1.2	1.4	1.7	2.0	2.5	3.5

such experiments by Kimball (1937) and Sonneborn (1937, 1938a and unpublished). In variety 1, the ratio of type II to type I varies from 0.5 to 3.4 in different experiments, though most of the experiments—as indicated by the larger number of caryonides—give ratios close to 1.0 and 2.0. Likewise, in variety 2, the ratio of type VI to type V varies from 0.9 to 3.5; in this variety, the absence of experiments with a great excess of type V and the absence of any tendency for the ratios to be simple are conspicuous.

What is the basis of these diverse ratios? They do not depend entirely on differences in the genetic constitution of the parents, for the same parent clones gave different ratios in different experiments. Further investigation showed, as appears in Table 7, that the temperature during

TABLE 7

P. aurelia. Stocks with two mating types.
Effect of temperature on inheritance ratios at conjugation.

Variety 1				Variety 3			
Temperature	Number caryonides		Ratio II/I	Temperature	Number caryonides		Ratio VI/V
	Type I	Type II			Type V	Type VI	
10°–19° C	87	80	0.9	10°–14° C	138	153	1.1
30° C	29	50	1.7	18°–30° C	128	278	2.2

Variety 3				
Temperature during		Number of caryonides		Ratio VI/V
Conjugation	Reorganization	Type V	Type VI	
10°	10°	66	64	1.0
20°	10°	50	44	0.9
10°	20°	27	62	2.3
20°	20°	31	62	2.0
20°	30°	29	64	2.2
30°	20°	21	73	3.5
30°	30°	36	96	2.7

conjugation and subsequent nuclear reorganization is one important factor. Both the ratio of type II to type I and type VI to type V is twice as great at higher temperatures as at lower ones.

Further analysis, in variety 3, throws some light on the period of nuclear activity that is sensitive to the different temperatures. The low ratio characteristic of low temperatures appears when the temperature is 10° C. during the period of nuclear reorganization (*i.e.,* between the time that conjugation is completed and the time that each enconjugant undergoes its first fission) and is independent of the temperature during conjugation itself. On the other hand, the high ratios obtained at high temperatures appear when the temperature during conjugation is 30° C., regard-

less of what it is thereafter. It appears likely that factors other than temperature also influence the mating type ratios.

The temperature effective period in all these experiments ends with the first fission after nuclear reorganization. By this time the mating type has become fixed; no changes are producible by further changes of temperature. The type previously determined is inherited absolutely through all succeeding fissions until the next nuclear reorganization. As the temperature has been a factor in determining the mating type during nuclear reorganization, we have here a demonstration of the effectiveness of an environmental condition in determining an inherited character. From the evidence presented above, demonstrating that the mating types are determined directly by the macronucleus, it is to be inferred that the environment acts on the macronucleus so as to "set" it for one type, and that when so set, it reproduces thereafter true to this type independently of later environmental conditions. In agreement with this, the temperature effective period is the period in which the macronuclei and the micronuclei that give rise to them are developing. In this striking example of the inheritance of environmental effects, we are dealing with inheritance through a type of nucleus peculiar to the ciliate Protozoa, the macronucleus, which may be characterized as a purely somatic nucleus, persisting and active only during vegetative reproduction and disappearing at conjugation and uniparental nuclear reorganization. These environmental effects therefore are not passed on through sexual reproduction, but only through vegetative reproduction and are of significance only in uniparental genetics, not in biparental genetics.

Results of the kind just set forth appear likely eventually to lead to a better understanding of one of the most perplexing chapters in protozoan genetics. In an extensive series of investigations on Paramecium, Jollos (1921) developed the concept of "Dauermodificationen," subsequently extended to other lower and higher organisms.

These long-lasting environmentally induced modifications, persistent sometimes through hundreds of fissions, were observed to appear and disappear and sometimes to reappear at conjugations and uniparental reorganizations. In some respects, the parallel to the phenomena of mating types is striking: these too appear, disappear and reappear at nuclear reorganizations; and, further, they are subject to environmental influences at the time of nuclear reorganization.

The fact that environmental conditions at the time of nuclear reorganization influence the relative frequency with which the two mating types are produced might lead one to suppose that the two caryonides from a single reorganized individual would nearly always be of the same mating type, for this is determined by the two macronuclei produced in the same cell at the same time and within 100 micra of each other. Experiments, however, flatly contradict this supposition. As a rule, there is no special tendency for the two macronuclei formed in the same individual to be alike: they are alike only so often as one would draw two red or two black cards from an infinitely large deck that contained red and black cards in the ratio in which the two mating types occur. In other words, the relative frequency of the three possible kinds of results (both caryonides type I, both type II, or one of each type), is determined solely by chance, that is, by the relative fre-

TABLE 8

P. aurelia, stocks with mating types I and II. Typical experiments showing that the mating types of the two caryonides from each reorganized individual are independently determined.

Ratio of type I caryonides to type II caryonides	Number of recognized individuals					
	Both caryonides type I		One caryonide type I, one type II		Both caryonides type II	
	Observed	Theoretical	Observed	Theoretical	Observed	Theoretical
2 : 1	42	41.3	40	41.3	11	10.3
1 : 1	35	34.8	70	69.4	34	34.8
1 : 2	22	19.7	76	78.8	79	78.8
1 : 3.4	6	4.5	28	30.9	54	52.6

quency of the two types. In Table 8 appear the observed and theoretically random values for representative diverse mating type ratios: 2:1, 1:1, 1:2, 1:3.4. The agreement between observation and theory is always close.

Ordinarily the same relations are found in variety 3 (mating types V and VI) as in variety 1: the two caryonides produced by a single reorganized individual are no more often alike in mating type than would be expected by chance alone. Nevertheless, in at least one stock (Q) of this variety the two caryonides from each exconjugant are nearly always of the same mating type, providing conjugation and the immediately following nuclear development have taken place at 10° C. Moreover, from the type V and VI individuals that conjugated, there arise two caryonides of type V from the former and two of type VI from the latter: under these conditions, therefore, the mating type ordinarily does not change at conjugation. If, however, the temperature is 20° during conjugation and 10° during nuclear development or 10° during conjugation and 20° during nuclear development, the two caryonides of an exconjugant are alike only so often as expected by chance; only when *both* these processes occur at 10° C. are the caryonides from an exconjugant regularly alike. The temperature at certain periods thus determines not only the mating type ratio, as earlier set forth, but also the *method of inheritance.*

I turn now to those instructive studies of Kimball on inheritance of mating type within the single caryonide. Ordinarily, as Sonneborn (1937) found, and Kimball (1937) confirmed, the mating type is strictly inherited within the caryonide. But Kimball (1939a) found in stock S that when animals of type II undergo uniparental nuclear reorganization and are transformed into type I, the change to type I does not occur at once and occurs at different times in different progeny of the reorganized individual. As a consequence, there are present in a single culture at the same time some individuals of type II and some of type I, so that conjugation can and does occur within such a culture. Kimball demonstrated beautifully

that each pair of conjugating individuals in such a culture consists of one individual of type I and one of type II, in spite of the fact that both are members of the same caryonide.   For, if the members of such a pair of conjugants are experimentally separated before fusion has become tight, the one member behaves like type II: it gives the sex reaction when introduced into a culture of type I, not when introduced into a culture of type II; and the other member does just the reverse: it behaves like type I, reacting with type II, not with type I.   Kimball further showed that though these individuals are phenotypically different in mating type, they are genotypically the same.   For if each is allowed to multiply by fission, the cultures produced by both of them are found to consist exclusively of individuals of type I.   The individual that had behaved as type II gave rise to progeny of type I only!   In such caryonides, the occurrence of individuals of type II is thus just a briefly transient phase; they are found only among the first individuals produced in the caryonide.   As this phenomenon appears only when the caryonide is genotypically type I and arose at nuclear reorganization from ancestors genotypically type II, the ancestral phenotype persists for a short time after the genotype has changed.   The same ''lag'' in change of phenotype following a change of genotype was observed in the inheritance of other characters by Sonneborn and Lynch (1934) and De Garis (1935). From our present point of view, it illustrates that within a caryonide individuals that are all alike in nuclear constitution may transiently differ in phenotype and that individuals of one phenotype may produce descendants of the opposite phenotype in the absence of any further nuclear reorganization.   This is a very special though important case and could be discovered only by careful search, for it appears only during the first few days after nuclear reorganization and occurs only when the mating type has been changed from II to I, not when the reverse change from I to II takes place.   I have confirmed this observation of Kimball's in other stocks of variety 1 and in variety 3.   In the latter, the ''lag'' occurs when the

mating type changes at nuclear reorganization from VI
to V, but not during the reverse change.　It occurs at con-
jugation as well as at uniparental nuclear reorganization;
but in some stocks it can not be detected at conjugation,
namely, in those stocks showing a period of immaturity,
for in these the immature period (7 to 10 days) is longer
than the lag period.

In another paper, Kimball (in press) reports a much
rarer but even more significant phenomenon.　In about 3
per cent. of the caryonides examined in stock S, the mating
type changed repeatedly in both directions; animals of type
I produced some progeny of type II, for their descendants
conjugated among themselves; and animals of type II pro-
duced some progeny of type I, for their descendants also
conjugated.　Both of these changes of type were later re-
versible.　Kimball showed that the two types were actu-
ally present both by the fact that conjugation occurred and
also by direct tests of mating type on the two individuals
of pairs that had been experimentally separated after com-
ing together to start conjugating.　He also demonstrated
that these repeated changes of type occurred in the absence
of new nuclear organizations.

The chief importance of these observations for our gen-
eral understanding of the phenomena of mating types lies
in the fact that these very rare "unstable" caryonides of
P. aurelia appear to be similar in behavior to the usual
type in certain other species.　For example, in certain
races of *Blepharisma undulans* (Woodruff, 1935) the vege-
tative progeny of any individual can conjugate among
themselves.　The question arises therefore as to whether
this is to be understood as evidence for the absence of
mating types—for the ability of any individual to mate
with any other individual.　In *P. aurelia,* where such phe-
nomena occur as rare exceptions, Kimball's conclusive
analysis shows that conjugation does not occur between
any two individuals, but only between individuals differing
in mating type.　This suggests that similar relations may
exist also in species like Blepharisma; in these species un-

stable caryonides may be the predominant or only kind present.

(2) *Stocks that contain only one mating type.* In stocks in which only one mating type appears, the genetic phenomena are much simpler. No conjugation occurs *within* such stocks, for only one type is present. When uniparental nuclear reorganization occurs, no change of type takes place. Inheritance is absolute both through vegetative reproduction and nuclear reorganization. The mating type of these stocks is always type I; stocks containing type II only have not been found.

(3) *Hybrids between stocks containing type I only and stocks containing both types I and II, and the progeny of these hybrids.* As will appear, genetic analysis of hybrids between the two kinds of stocks in variety 1 led to the discovery of clear-cut Mendelian inheritance: the first discovery of inheritance in Mendelian ratios in the ciliate Protozoa.

The characters dealt with here are the one-type condition, in which clones remain permanently type I, never changing at nuclear reorganization; and the two-type condition, in which both types I and II appear within a clone. In the latter condition, the two types reproduce true to type only during vegetative reproduction, either mating type yielding caryonides of both types after nuclear reorganization.

From 149 pairs of hybrids between stocks showing these diverse conditions, *all* the caryonides produced by the hybrid exconjugants showed the two-type condition. Hence, the two-type condition is dominant over the one-type condition. If the parent stocks differed in a single pair of genes, the two-type parent stock may be represented as of genotype AA, the one-type parent stock as aa, and the F1 hybrids as Aa.

The hybrids (Aa) were then backcrossed to the recessive parent (aa). Of the resulting pairs of conjugants, half should be of genotype Aa and show the two-type condition and the other half should be of genotype aa and show the one-type condition. In this backcross, 158 pairs of con-

jugants were obtained: in 81 of the conjugant pairs, each exconjugant produced two caryonides showing the two-type condition, and in 77 of the conjugant pairs, each exconjugant gave two caryonides showing the one-type condition. Thus, the backcross yielded the typical Mendelian result for a single gene difference: approximately equal numbers of dominants (81) and recessives (77).

The hybrids (Aa) were further tested by interbreeding them to get an F2 generation composed of 120 pairs of conjugants. In 88 of the pairs each exconjugant gave two caryonides showing the two-type condition and in 32 of the pairs each exconjugant gave two caryonides showing the one-type condition. This is very close to the expected Mendelian ratio of three dominants to one recessive.

Of the F2 dominants, one-third should be homozygotes (AA) and two-thirds heterozygotes (Aa). This was tested by crossing nineteen of the F2 dominants to the recessive parent stock (aa). Six of the F2 dominants crossed to the recessive yielded only dominant progeny; they must, therefore, have been homozygotes (AA) and their progeny heterozygotes (Aa). The remaining 13 F2 dominants yielded one-half dominants and one-half recessives; they must therefore, have been heterozygotes (Aa) and their progeny half heterozygotes (Aa) and half homozygous recessives (aa). Hence, the F2 dominants included both homozygotes and heterozygotes in the proportion expected.

The foregoing results supply the strongest genetical evidence that genes and nuclei are exchanged between the two conjugants of a pair. Though this has long been accepted on cytological grounds, it has in recent years been challenged by Diller (1936) and by Wichterman (1937) who hold, on the basis of cytological observations, that exchange of pronuclei between conjugants may not occur in certain species of Paramecium. On the genetical side, the common genetic similarity between two individuals that have conjugated together was first discovered by Jennings (1913), whose observations have many times been confirmed, recently, in a striking way by Sonneborn and

Lynch (1934) and by De Garis (1935). The Mendelian analysis set forth above puts this type of observation on a new footing: the genic agreement between the two conjugants of a pair and the relative frequencies of the various gene combinations permit no doubt that in these stocks the nuclei of the conjugants undergo reductions and cross combinations according to the classical scheme.

Uniparental nuclear reorganization in hybrids. Turning now to inheritance in hybrids of genotype Aa at uniparental nuclear reorganization, the results are very different from those in either of the parental stocks. When a hybrid exconjugant is allowed to multiply and the progeny undergo uniparental nuclear reorganization, the reorganized individuals are found to yield progeny of two kinds: half of them show the two-type condition and half show the one-type condition. By appropriate breeding tests it was shown that the former are homozygous dominants (AA) and the latter homozygous recessives (aa). In subsequent uniparental reorganizations, no further changes of genotype occur.

How can such results be accounted for? If, at nuclear reorganization, the chromosome number is reduced to the haploid condition and this is followed by an equational division to form two gamete nuclei which fuse, the genetical results observed would occur. Thus, in heterozygotes (Aa), reduction would give in half the animals A and in half a; subsequent formation of gamete nuclei and syncarya would transform the former half into AA, the latter into aa.

These cytological processes are essentially those held to occur at uniparental nuclear reorganization by Diller (1936). The genetical evidence thus agrees with his cytological account of autogamy. If the non-autogamous endomixis described by Woodruff and Erdmann (1914) had taken place, the heterozygotes should have remained heterozygotes. Thus far, this has not been observed in a single case. However, the observations of Woodruff and Erdmann were carried out mainly, as is now known, on

variety 2, while the preceding analysis was made on variety 1 of *P. aurelia*. Whether genetic evidence of non-autogamous endomixis can be obtained in variety 2 or under other conditions in variety 1 remains to be determined.

The significance of autogamy in the biology of *P. aurelia* is great. It explains why natural stocks are all homozygous after a short time in the laboratory. The heterozygotic condition can endure only during the few weeks that intervene between a cross and the first nuclear reorganization in the hybrids. Thus *P. aurelia*, though a diploid organism, is ordinarily homozygous. There are no hidden recessive genes. For genetical analysis therefore, they are as favorable as haploids.

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PARAMECIUM BURSARIA: MATING TYPES AND GROUPS, MATING BEHAVIOR, SELF–STE–RILITY; THEIR DEVELOPMENT AND INHERITANCE[1]

DR. H. S. JENNINGS

As you will see, the situation in *Paramecium bursaria* differs in important ways from that in *Paramecium aurelia,* giving indeed a very different system.

Paramecium bursaria, the green Paramecium, is somewhat less familiar to many of us than *Paramecium aurelia* or *caudatum.* Yet it occurs commonly and abundantly in our fresh waters.

In this species too we find that members of the same clone ordinarily do not conjugate together, yet if two clones of diverse origin are mixed, we may get the immediate spectacular clumping and pairing that were shown in the demonstrations. The two clones belong to diverse mating types.

This clumping or agglutination is very like that which occurs in *Paramecium aurelia;* and also like that which occurs in mixtures of the two different types of gametes of some of the lower algae. In Paramecium the individuals are so large that their behavior in this agglutination can be readily observed. I should like to say a few words about some of the more striking features of the behavior.

When individuals of different mating type (but of the same "group") are mixed, it is to be observed that the individuals are not pulled together or guided toward each other from a distance. They continue to swim about in the usual way. But these movements of many individuals in a limited space soon bring some of them into accidental contact. An individual of one of the types brushes against

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one belonging to the other type. Thereupon the two stick together, exactly as if their surfaces were covered with some strong adhesive material. There is no definite reaction, no coordinated movements of the two. Each of the individuals tries (as it were) to continue moving as before, but as they stick together, both are suddenly stopped, or one drags the other against the motion of the latter's cilia; or if the two anterior ends happen to be in the same direction, the two swim forward together.

Any parts of the body that thus come in contact adhere. The two individuals may come in contact by their aboral surfaces or their rear ends or in any irregular way; thereupon they adhere, and begin to move in an irregular manner resultant upon the divergent action of their free cilia. Often one individual drags another backward or sidewise through the water.

Two individuals thus stuck irregularly together flounder about in the crowded drop, and soon come into contact with other individuals of one or the other type. A third individual adheres irregularly to the two, then a fourth, and this continues until large masses are formed, containing twenty to a hundred individuals or more. In these the individuals are in irregular contact, by any parts of the body. In all this it is evident that the coming together of the individuals is the accidental result of their ordinary motions; they adhere when thus accidentally brought together. Until the individuals come into contact there is no indication of stimulation or of a change in behavior.

In the mixtures containing many individuals of the two types, groups are formed with all sorts of irregular attachments. Within three or four minutes after the two types are mixed, large masses of adhering individuals are formed. Such masses may contain hundreds of individuals. The individuals in the clot adhere firmly together by whatever parts of the body are in contact. They do not move freely with relation to each other, being held seemingly by a rigid physical adhesion. By using two types that differ in depth of color or in other marked ways, it can be seen that the adhesion is always between individuals

belonging to the two different types. The large masses contain thus about equal numbers of the two types.

The masses commonly remain firmly united for about half an hour, then begin slowly to break up into smaller masses. Yet masses of considerable size may remain united for two hours or more. As the masses break up, many longitudinal chains are found to have been formed, the individuals (alternately of different types) being united end to end.

Even in the tightly adhering groups the individuals may shift slightly, moving slowly and with seeming difficulty. In this way the relative positions become slowly changed. Some of the individuals come thus in contact by their oral surfaces. There is now apparently some coordinated motion, till the two individuals come into the typical mating position. This is a slow process, requiring usually an hour or more. As the large masses slowly disintegrate, it is found that many of the individuals have become united in pairs.

In *Paramecium bursaria,* as in *Paramecium aurelia,* the clumping and pairing are dependent on the time of day. In most types they take place most strongly about midday and for three or four hours after. About 5 o'clock P.M. or thereabouts the clumping becomes weaker, and by six or seven it has entirely ceased, and the clots earlier formed completely disintegrate. However, the time scheduled for the reactions differs in the different groups: in one group (Group III), the reaction may occur at any hour of the day or night.

We will now return to the mating types themselves. The phenomena that we have described show that in *Paramecium bursaria,* as in *Paramecium aurelia* there are two mating types. Members of a single type do not conjugate together, but when the two are mixed, clumping and conjugation occur. We may call the two mating types that we have discovered A and B.

Now as we test other clones, we find some that conjugate with A, others that conjugate with B. And as we con-

tinue our testing, we come upon a clone that conjugates readily with *either* A or B.   We have found a third mating type, C, whose members do not conjugate together, but do conjugate with both A and B.

Continuing to collect and test clones, we find one that conjugates readily with any of the three—with A or with B or with C.   We have a fourth mating type, D.

No matter how long we continue to collect and test clones we never find an additional mating type in this set.   There are just four and no more.   The relations between A, B, C and D are shown in Table 1, in which a plus sign indicates the occurrence of conjugation.   The members of any type, as A, do not conjugate together, but do conjugate readily with members of any of the other 3 types.   Every clone of this group belongs to one of the four types.

TABLE 1

	A	B	C	D
A	−	+	+	+
B	+	−	+	+
C	+	+	−	+
D	+	+	+	−

But as we continue to collect and test clones from different regions, we find some that do not conjugate with A, B, C or D, but do conjugate together when mixed.   These then form a different group.   The clone E conjugates with the clone F, though not with A, B, C or D.   We continue to collect clones, and find one whose members conjugate with both E and F.   We now have three mating types in our second group, E, F and G.   We continue in this manner, adding new types in this group until we have eight.   And there we stop; no more are to be found.   We have one group in which are four mating types, A, B, C and D; another in which there are eight, E to M.   In this second group, as in the first, the members of any type do not con-

jugate together, but do conjugate readily with members of any of the other seven types. The relations of the types in this group are shown in Table 2.

TABLE 2

	E	F	G	H	J	K	L	M
E	−	+	+	+	+	+	+	+
F	+	−	+	+	+	+	+	+
G	+	+	−	+	+	+	+	+
H	+	+	+	−	+	+	+	+
J	+	+	+	+	−	+	+	+
K	+	+	+	+	+	−	+	+
L	+	+	+	+	+	+	−	+
M	+	+	+	+	+	+	+	−

Each type includes many diverse clones. As we collect a new clone, we determine to what type it belongs by testing it with members of the known types. Suppose it turns out to belong to Group II. It is tested successively with the eight types of that group. With seven of them it forms pairs. But invariably it selects one of the eight with which it does not react; with which it never clots nor forms pairs. This therefore is the type to which it belongs; it does not mate with members of its own type. As one tests hundreds of clones gathered from many parts of the United States, it appears astonishing that each one selects as it were one type with which it refuses to conjugate, while with all the others of its group it does conjugate. Of the mating type E there are now known 132 diverse clones collected from different parts of the country—from Maryland to California. Of the mating type F, 29 clones are known, and so on.

As we continue tests we come upon another group of clones that do not form pairs with any of the members of Group I nor of Group II, but do form pairs when two of its clones are mixed together. We have therefore found a third independent group. And as we study these, we find that this Group III, like Group I, contains just four mating

types, N, O, P and Q, which show to each other the relations seen in Table 3.

TABLE 3

	N	O	P	Q
N	−	+	+	+
O	+	−	+	+
P	+	+	−	+
Q	+	+	+	−

Thus, so far as investigation has now gone, the species *Paramecium bursaria* consists of three independent groups, the members of any one group not conjugating with the members of the other groups. The constitution of the species is therefore that which is shown in Table 4.

TABLE 4

A	B		E	F	G	H		N	O
C	D	......	J	K	L	M	......	P	Q

Group I and Group III each contain four mating types, Group II eight mating types, so that there are in all sixteen diverse mating types. The mating relations of the 16 types are shown in Table 5.

The geographical distribution of these 3 groups and 16 mating types is of interest. The 4 types of Group I have thus far been found only in Maryland and Virginia, but I shall be surprised if they are not ultimately found all over the United States. The eight types of Group II have been found from Cape Cod, Massachusetts, in the East, to California in the West; from East Aurora, New York, in the North, to North Carolina in the South. Group III have been collected on the one hand from near Pineville, North Carolina, on the other hand from about Provincetown, at the tip of Cape Cod, Massachusetts. It would not be surprising if it turns out that all three groups are generally distributed on the North American continent. As to other continents, nothing is known. Possibly other groups and types will be found.

TABLE 5

		I				II								III			
		A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q
I	A	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	B	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
	D	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
II	E	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−
	F	−	−	−	−	+	−	+	+	+	+	+	+	−	−	−	−
	G	−	−	−	−	+	+	−	+	+	+	+	+	−	−	−	−
	H	−	−	−	−	+	+	+	−	+	+	+	+	−	−	−	−
	J	−	−	−	−	+	+	+	+	−	+	+	+	−	−	−	−
	K	−	−	−	−	+	+	+	+	+	−	+	+	−	−	−	−
	L	−	−	−	−	+	+	+	+	+	+	−	+	−	−	−	−
	M	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−
III	N	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	O	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	P	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+
	Q	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−

The three groups seemingly remain quite independent, never intercrossing. Hundreds of tests have been made by intermixing members of the different groups, but no pairing has occurred.

The three groups are not distinguishable by any well-marked morphological characteristics. Within each group, and indeed within each of the 16 mating types there are many different clones, and these clones may differ from each other greatly in size and form. These differences among the clones of even a single type are so much greater than any differences characteristic of the types or groups that the latter are difficult to detect and perhaps do not exist. There are, however, some physiological differences among the groups. Groups I and II show the mating reactions only during certain hours of the day, while in Group III the reactions occur at any hour of day or night.

And here we must introduce one of the exceptional conditions that soften the hard outlines of the system. Ordinarily, as we have said, the members of a single clone do not conjugate together; they all belong to the same mating type. To obtain conjugating pairs we must mix together clones of two diverse mating types.

But in rare instances one comes upon one or more pairs in a culture of a single clone. This is so rare that one may study particular clones for months and see no examples of it. But in the course of time instances of such self-fertilization of a clone accumulate. Individuals are found that adhere to each other in the way earlier described in the clumping of diverse types; such individuals finally pair completely, exchanging nuclei in the usual way, and producing clones of descendants.

Has the single clone before conjugating differentiated into two mating types? This question is to be answered by isolating the two individuals that cling together and determining by tests whether they belong to the same or to diverse mating types.

This was done in the case of a clone that originally belonged to type D of Group I. A considerable number of pairs appeared in this. Tests for the differentiation into diverse types were carried out as follows. Two individuals that adhere in the way that is a preparation for conjugation are separated before they have become really united, and these are tested separately with members of the four mating types. Also, in a considerable number of cases (eighteen in all), the two individuals thus separated were allowed to multiply till each had produced a numerous clone. The two clones were then tested with the four mating types. In every case it was found that the two individuals that had initiated conjugation did indeed belong to diverse mating types. One belonged invariably to type D, the other invariably to type A. The clone was originally of type D. In some way some of its members have become differentiated into type A; the two types then conjugate. As Sonneborn showed in the paper preceding this, in

Paramecium aurelia such a differentiation into two types may occur at the time of the nuclear reorganization known as endomixis. In *Paramecium bursaria* endomixis does not occur at regular intervals; it is indeed extremely rare. Erdmann (1927), who studied this matter, could not find endomixis in isolation cultures at all, but believed that she did find evidence of it in mass cultures. It appears probable therefore that these rare cases of self-fertilization of clones are a consequence of the equally rare occurrence of endomixis. To determine this with certainty will be difficult, because of the extreme rarity of both phenomena. But the observations that I have described show that in the cases of self-fertilization of a clone, we are really dealing as usual with the conjugation of individuals that belong to two diverse mating types.

This brings us to the development and determination of the different mating types. How is it determined to what mating type shall belong a particular individual and the clone that it produces by fission?

Here the first question for examination is: What is the relation of the mating type in the descendants to the mating type of the parents? For ordinary fission the answer to this question is very simple. All the descendants by simple fission of an individual are of the same mating type as the parent individual. The mating type is strictly and simply inherited in ordinary fission.

The only apparent exceptions to this are the rare cases of self-fertilization of a clone, just described. In such cases presumably, as we saw, some special nuclear process has occurred in addition to simple fission. But this matter remains to be investigated.

What do we find as to the inheritance of the mating types at conjugation? The two members of a pair of conjugants —which I shall call the two parents—are always of different mating type, as two parents in higher organisms are always of different sex. What is the relation of the mating type in the descendants of the pair to the two types represented by the parents?

In ourselves and other higher organisms only one combination of the mating types is possible; male with female. But in our infusorian with its many different types, many diverse combinations may occur.   In Group I, with its four mating types, A, B, C, D, six different crosses are possible, namely, $A \times B$, $A \times C$, $A \times D$, $B \times C$, $B \times D$ and $C \times D$.   In Group II with its eight types, the number of diverse possible crosses is $\frac{1}{2}$ (7×8), or 28.   In Group III, again 6 diverse crosses may occur.   Thus the total number of possible different crosses is 40.

Only a few of these 40 crosses have yet been examined. In Group I all the six possible crosses have been made and to some extent studied, so that I shall set forth only the relations found in that group.

The plan of study is as follows.   In each pair the two parents are of diverse mating type, for example, A and D. They conjugate, exchanging halves of their micronuclei, then separate.   Each is now allowed to divide into two, and these two are cultivated separately, so that each produces a clone.   Thus four clones are produced from each pair, two from each ex-conjugant.   For each of these four clones the mating type is discovered, so far as possible, by testing with the four known types, A, B, C, D.

The first thing that one learns in such tests is that for a considerable time after conjugation the descendants of the ex-conjugants do not conjugate at all, with any of the mating types.   There is a period of immaturity which lasts usually for some months.   The different clones descended from union of the same two mating types vary greatly in the length of the period of immaturity.   Many of the ex-conjugant clones of Group I, from a conjugation of June 8 last, are still immature, more than six months after the conjugation.   Others have become mature at varying intervals.   The shortest period observed for the beginning of mating was twenty-two days from the parental conjugation.   But for most clones immaturity lasts for months.

Complete maturity does not come on suddenly.   There is a long period of partial immaturity, of "adolescence" in

which the mating tendency is weak and but few individuals
of the clone conjugate.   In a test mixture of a hundred or
more individuals from each of two clones, frequently but
one or two pairs will be formed, and these only after the
two clones have been together for several days.   This
makes much difficulty in determining the mating type of
young clones.   In Group I, the mating type has thus far
been determined by test for 250 young clones, derived from
164 ex-conjugants, belonging to 116 different pairs.

A third thing discovered in the tests is a matter of much
interest.   We discover that at first the young clones differ-
entiate into two types only, not into the final four.   One of
these two types conjugates with the adult types C and D,
but not with types A nor B.   On our usual principles it may
be called the type $\overline{AB}$.   The other type conjugates with the
adult types A and B, but not with C nor D.   It will have
to be called the type $\overline{CD}$.   Thus in some of the pairs at a
certain period all the four clones descended from the pair
are of the type $\overline{AB}$; in others all the four clones from a pair
are of the type $\overline{CD}$.

These two types $\overline{AB}$ and $\overline{CD}$ differentiate farther in the
course of time, so as to form the four adult types A, B, C
and D.   That is, some of the individuals of the type $\overline{AB}$,
which at first mate only with C and D, in time acquire the
power to mate also with type B, but not with type A.
These have therefore developed from type $\overline{AB}$ to type A.
Those from some other pairs develop into type B.   In the
same way the clones of type $\overline{CD}$ derived from some pairs
develop into type C, while those from other pairs develop
into type D.

Thus the four adult types A, B, C, D are slowly developed
from the immature individuals, which first differentiate
into two types; then each of these two develops into two
further types.   The types A and B are more closely re-
lated to each other than either of them are to C or to D.   A
and B are closely related, also C and D are closely related.

The offspring have therefore after some weeks or
months reached the adult condition, in which there are four

diverse mating types, A, B, C, D. We may now compare
the final types in the offspring with the types in the par-
ents. First, as to general relations: Table 6 shows those

TABLE 6

Group I

Four Types: A, B, C, D

Six Possible Crosses: A × B, A × C, A × D, B × C, B × D, C × D

Mating Types Given Thus Far by the Six Crosses (Conjugation of June 8, 1938, or Earlier):

Cross					
A × C	yields	A, B,	C,	D	
A × D	"	A, B,	C,	D	
B × C	"	A, B,	C,	(D?)	(Many immature)
B × D	"	A, B,	(C?),	D	(Many immature)
A × B	"	A, B			(Most still immature)
C × D	"	C, D			(Most still immature)

thus far observed. A certain cross of two parents, as
A × C, gives rise to all the four types A, B, C, D. This is
true also for the cross A × D; and probably also for two
others, B × C and B × D, though these are not fully worked
out yet, most of the clones being still immature. The re-
maining two crosses are those between the two closely re-
lated types, A × B and C × D. Each of these pairs of types
were, as you remember, originally united in one type $\overline{AB}$
or $\overline{CD}$. The crosses of these two related types have thus
far given only the two parental types. But the data are
incomplete; most of the young clones from these crosses
are still immature.

Coming now to specific and numerical relations between
the parental types and those of the descendants, we dis-
cover the relations shown in Table 7, which gives the results
for the crossing of type A with type D.

TABLE 7

CROSS, TYPE A BY TYPE D. 31 PAIRS: FOUR CLONES FROM EACH PAIR, TWO FROM PARENT A, TWO FROM PARENT D

	From parent A	From parent D
13 pairs gave	A, A	A, A
14 " "	D, D	D, D
2 " "	B, B	B, B
2 " "	C, C	C, C

The first important fact to be observed is that in almost

all cases—in *all* cases in this cross A × D—the four clones derived from a given pair are alike in their mating type; all four are A, or all are D, or all are B or C.

And second we find that in the great majority of cases the type to which the four belong is the same as that of one of the two parents. In the cross of A × D, shown in Table 7, 27 of the pairs gave descendants that were all like one of the two parents—like either A or D, while four did not. About half the pairs give descendant clones that belong to one of the parental types, the other half to clones that belong to the other parental type. In the case of the cross just mentioned, there were 31 pairs from parents that were A and D. Of the 31, thirteen gave descendants that were all of type A, while fourteen gave descendants that were all of type D.

A third important fact is shown in Table 7. A few of the pairs—four out of thirty-one in this case—give descendants that are not of either parental type. Two give clones that are all of type B, two others clones that are all of type C.

These relations appear typical for most of the crosses in Group I. Of the 250 ex-conjugant clones fully tested, 205, or 82 per cent., belong to one of the two parental types, while 45, or 18 per cent., are unlike the parents in type.

How can these relations be accounted for? Leaving out of account for the present the few exceptional cases, the reappearance of the two parental types in equal numbers in most of the descendants may be accounted for by conditions similar to those which result in the production of equal numbers of the two sexes in higher organisms. Suppose that the parental types are diverse in a single chromosome or gene, so that one type is XX while the other is XY. Then by their union we shall get again in the next generation equal numbers of XX and XY; that is, the two parental types.

But why should all the descendants of a particular pair be alike in their mating type? This is much as if all the

members of a family of children should be alike in sex: all
males or all females.

This seems to result from certain special conditions that
exist in the ciliate infusoria. During conjugation the
micronucleus of each ex-conjugant divides twice, yielding
four pronuclei. Three of these disappear, leaving but one.
This divides again—the so-called third division—and one
of the two products is exchanged for one from the other
member of the pair.

If the nucleus of one individual contains $\underline{XX}$, while that
of the other contains $\underline{XY}$, then if reduction occurs at either
the first or second division, the four nuclei from the parent
$\underline{XX}$ will be $\underline{X}$, $\underline{X}$, $\underline{X}$, $\underline{X}$, while from the parent $\underline{XY}$ the four
will be $\underline{X}$, $\underline{X}$, $\underline{Y}$, $\underline{Y}$. Now three of the four disappear in each
case, leaving but one. In the parents $\underline{XX}$ will remain the
single nucleus $\underline{X}$, while in the cases of parents $\underline{XY}$ there
will be left in half the cases the single nucleus $\underline{X}$, in the
remaining cases the single nucleus $\underline{Y}$.

Notice first the cases in which each of the two parents
now contains the single nucleus of constitution $\underline{X}$. Next
occurs the third division of the nucleus, after which each of
the two parents obviously contains two pronuclei, $\underline{X}$, $\underline{X}$.
They exchange one of these, after which it is evident that
each parent contains the nuclear combination $\underline{XX}$. The
two parents are therefore alike, both being of the type $\underline{XX}$;
their descendants by vegetative fission will therefore all
belong to the single type $\underline{XX}$.

In the other possible case, after reduction at the first or
second division with disappearance of three of the four
nuclei, one parent contains the single nucleus $\underline{X}$ the other
the single nucleus $\underline{Y}$. After the third division one has two
pronuclei $\underline{X}$, $\underline{X}$, the other two pronuclei $\underline{Y}$, $\underline{Y}$. Now occurs
the exchange of one pronucleus, after which each of the
two parents obviously contains the combination $\underline{X}$, $\underline{Y}$.
Again the two parents are alike and both are of the type
$\underline{XY}$; their vegetative descendants are therefore all of the
single type $\underline{XY}$.

If however reduction did not occur until the third nuclear

division, the parent XY would contain two final pronuclei, one of which would be X̲, the other Y̲. The result would be that one of the two ex-conjugants would receive the combination XX̲, and all its descendants would be of that type, while the other ex-conjugant of the same pair would contain XY̲, and all its descendants would be of that type. The descendants of the two ex-conjugants of such a pair would be of diverse mating types. The fact that in almost all cases they are of the same mating type is perhaps strong evidence that reduction usually occurs at the first or second division of the micronucleus, not at the third division.

But in a few cases, the descendants of a single pair are not all alike in type. Among forty-eight pairs in which the type of the descendants of both ex-conjugants was determined, there were six pairs in which two different types occurred among the descendants. The mating types of the descendants of these six pairs are shown in Table 8.

TABLE 8

PARAMECIUM BURSARIA. SIX PAIRS (OUT OF 48) THAT GAVE DIVERSE MATING TYPES AMONG THE FOUR CLONES DERIVED FROM EACH PAIR

Parents	Mating types of the descendants from Ex-conjugant 1	from Ex-conjugant 2
A × C	A, B	B, B
A × C	D, B	D, D
A × C	A, B	?, ?
A × C	A, ?	C, ?
B × D	B, B	A, ?
C × (B?)	C, C	B, B

In three of the six pairs (the last three of the table) the descendants of the two ex-conjugants differed in type. This is the situation that would be brought about if in these three cases reduction had occurred exceptionally at the third division of the micronucleus instead of earlier.

But in three other cases, the descendants of a single one of the ex-conjugants included two types. One of the two clones resulting from the first division after conjugation was of one type, the other of a different type. In rare cases therefore the diverse mating types are segregated at the first division of the ex-conjugant.

There remain the cases in which the descendants of a

pair are all of a different type from either of the parents. As shown earlier, there were four such pairs from parents that were type A by type D. Two of these pairs gave 4 clones that were all of type B, the other two, four clones that were all of type C.

In considering such cases, it is to be remembered that the type B is derived with the type A from the single young type $\overline{AB}$, while type C is derived with type D for the single young type $\overline{CD}$. What happens then is this. When the parents are $A \times D$, all the young descendants of about half the pairs are of the type $\overline{AB}$, while the young descendants of the rest of the pairs are all of the type $\overline{CD}$. Then most of the $\overline{AB}$ develop into the parental type A, while a few develop into B. Similarly, most of those that are of type $\overline{CD}$ develop into the parental type D, while a few develop into the non-parental type C. The nature of the underlying factors and influence for these changes are as yet unknown.

The conditions that I have illustrated for the results of the cross $A \times D$ appear typical, so far as examination has gone, for the other crosses in Group I: but much remains here to be discovered.

And now a few words in conclusion as to the relation of the phenomena described to those found elsewhere in the world of organisms.

As you have seen, the conditions in *Paramecium bursaria* differ from those in *Paramecium aurelia* in the fact that in any race or group of aurelia there are but two mating types, so that the situation there resembles that in the higher animals and plants, with their two sexes. In *Paramecium bursaria*, on the other hand, each group contains four or eight mating types. The development shows, as I have already set forth, that at first there are but two types, and that each of these differentiates into two, making four. The fact that in Group II there are eight types suggests that in this group differentiation has gone one step farther, each of the four types differentiating into two to make

eight.   All this suggests that the condition with but two
types is the original one, from which the condition with
multiple types has developed.

In the flagellates, according to the magnificent investi-
gations of Moewus, there are in each species or race, as
in the races of *Paramecium aurelia,* just two mating types.
The flagellates are haploid, so that the cells of the two
mating types are closely comparable to the male and female
gametes of higher organisms.   Moewus therefore considers
the phenomena in the flagellates as strictly sexual, speak-
ing of the two types as the two sexes.   In the ciliates of
course the organisms are diploid, so that the different mat-
ing types are not directly comparable to male and female
gametes, but rather to the two types of somatic individuals
that constitute the two sexes in higher organisms.   Yet the
situation in the ciliates differs from that in the typical
bisexual organisms in the fact that in most of them both
members of the pair produce descendants.

The many diverse mating types in *Paramecium bursaria*
invite comparison with the facts of ''multipolar sexuality''
in certain fungi.   Yet the differences between the cases are
greater than their resemblances.   The sexually reacting
parts in the fungi are haploid, not diploid.   Further, in the
fungi the situation is such that usually a given mating type
or sex mates with only one other type out of four, whereas
in *Paramecium bursaria* each mating type mates readily
with any of the other types of its group.   The conditions
in the fungi differ in principle and in details from those
found in the infusorian.

The phenomena in *Paramecium bursaria* appear most
directly comparable with the so-called ''self-sterility'' of
some of the higher plants and animals.   The single clone
of the infusorian is comparable to the single self-sterile
plant, it ordinarily does not fertilize itself; its cells do not
unite in conjugation.   But the single clone, like the single
plant, may be fertilized by another; the cells of the single
clone unite in conjugation with those of other clones, giving
viable descendants.

But this is by no means the whole story, either in the infusorian or in the self-sterile plant. The further phenomena in the two cases show many parallels in their details. In some cases the self-sterile plant becomes at times self-fertile, giving viable offspring; this is true also, as set forth earlier, for the single clone of the infusorian. Again, the phenomenon is not strictly one of "self"-sterility merely, in either the plant or the infusorian. The single plant is sterile also with certain other plants, just as the single clone of Paramecium is sterile with certain other clones (those belonging to the same mating type). In most self-sterile plants the individuals can be divided into classes, such that those belonging to the same class are infertile together, while those belonging to diverse classes are fertile together. Such classes are comparable to the different mating types of *Paramecium bursaria.* In this infusorian the number of such types is definite and limited: there are four in each of Groups I and III, eight in Group II; while in *Paramecium aurelia,* as Sonneborn shows, there are but two in each group. The conditions in the infusoria emphasize the similarity and possible relationship between the phenomena of self-sterility and those of sexuality.

For the division of the infusoria species into groups that do not cross-conjugate, I have not found parallels in the accounts of self-sterility in higher organisms. Its real parallel appears to be the actual differentiation of a genus into species that do not cross. The groups may perhaps be considered slightly marked cases of differentiation into incipient species: possibly it will eventually appear best to give them different specific names.

STUDIES ON CONJUGATION IN PARAMECIUM MULTIMICRONUCLEATUM[1]

DR. ARTHUR C. GIESE

STANFORD UNIVERSITY

MAUPAS (1889) in his classical studies on conjugation of the protozoa showed that conjugation was dependent upon both internal and external conditions, but clear evidence of the nature of the internal factors came only recently with the conclusive studies of Sonneborn (1937, 1938) on *Paramecium aurelia* and Jennings (1938) on *Paramecium bursaria,* in which mating types were described for these two species. With a better understanding of the internal conditions, the effect of the external conditions on conjugation can be more effectively studied. It is probable that a solution of many of the problems of conjugation lies in a proper understanding of the interrelationships of both intrinsic and extrinsic factors. In this paper are recorded some experiments on the action of internal and external factors in the conjugation of *Paramecium multimicronucleatum* Powers and Mitchell.

STUDIES ON INTERNAL FACTORS

In experiments recently reported (Giese and Arkoosh, 1939) eight local stocks of *P. multimicronucleatum* were tested for presence of mating types. In seven of the stocks conjugation occurred among the progeny of a single individual. Stained samples revealed no endomixis during the period of divisions preceding conjugation, and it is known that endomixis occurs relatively infrequently in this species of *Paramecium* (Stranghöner, 1932). Therefore the animals should be of similar nuclear and genetic constitution. To test if differentiation occurs just preceding

[1] Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

union, animals were separated from one another when in incipient conjugation and cultures were grown from each of the animals of the pair. Progeny of each of the two showed no greater attraction for one another than for their own kind. Progeny of ex-conjugants showed the same behavior. The experiments are described in detail in the paper cited.

That the stocks tested are relatively constant in behavior with respect to mating character is indicated by the fact that one of the stocks was cultured for over five years without variation from the pattern whenever tested. Others have been tested for shorter periods of time but adequately to indicate that the behavior is not aberrant and temporary.

The eighth stock, however, showed no conjugation when tested in the manner used for the other stocks. This exceptional stock (number 2) was subjected to variations in environmental conditions which according to various authors are conducive to conjugation. At the end of a series of several hundred negative experiments conjugants appeared, and thereafter occurred regularly in mass cultures under the conditions conducive for conjugation in the other stocks (Giese, 1938a). Isolation cultures now demonstrated the presence of two types in this population —one (2C) in which progeny of a single individual did not conjugate with one another regardless of conditions, another (2E) in which progeny of a single individual conjugated with one another. But most interesting was the fact that individuals of 2C readily conjugated with individuals of 2E provided both were in the proper physiological state. Thus when one individual of 2E was introduced into a culture of 2C, immediate clumping occurred and a conjugating pair appeared later. When five animals of 2E were added to 2C, not more than five pairs appeared. When 50 of each, 2C and 2E, were mixed as many as 80 or more of the 100 conjugated.

As pointed out above, progeny of a single member of 2E conjugated among themselves but not as readily as with 2C, particularly when the number of generations subse-

quent to subculture was small. When individuals of 2E in incipient conjugation were separated from one another and cultures were grown from each animal, members of the subcultures were found to attract one another more intensely than do members of a given culture (Table 3, Giese and Arkoosh, 1939). This indicates a secondary segregation of mating types in this stock.

In stock 2 the excretion crystals present are larger than those of other stocks, therefore in mixtures members of this stock can be distinguished from other stocks. Attempts were made to determine if mixed mating occurs between stock 2E or 2C and each of the other seven stocks. While conditions were apparently optimal and conjugation occurred, matings between stock 2 and the other stocks was not observed, each individual mating with its own kind. This points to (1) the presence of several sets of mating types, or (2) a situation not comparable to that in *P. aurelia* or *P. bursaria*.

One may conclude from these experiments that (1) either mating type differentiation is lacking in some stocks of this species; or (2) in such stocks mating types differentiate during vegetative division; or (3) reversal of mating types occurs during vegetative division. Kimball (1938) has recently shown that the second case occurs rarely in *P. aurelia*. When adequate means for testing the phenomena are established in *P. multimicronucleatum* it should be possible to decide between these alternatives. It is possible that the situation which is the exception in *P. aurelia* is the rule in *P. multimicronucleatum* and that in the latter species clearly defined mating types are the exception. More data are necessary and experiments are in progress on a number of stocks from various other localities.[2]

Aside from mating type differentiation another internal factor—the "maturity" of a stock—conditions conjugation. Thus in some of the stocks of *P. multimicronucleatum* a certain time must elapse subsequent to conjugation before

[2] Since this paper was written well defined mating types have been found in both local and distant stocks.

mating is again possible. It is well known that ex-conju-
gants of many species of protozoa show low viability.
Experiments on some stocks of *P. multimicronucleatum*
have shown that conditions optimal for growth of vegeta-
tive individuals are often deleterious for ex-conjugants
and that survival may be increased by modification of the
conditions (*e.g.,* decrease in food concentration and culture
temperature). "Maturity" may be bound up with the
recovery of a "proper" physiological state.

Studies on Environmental Factors

While proper internal conditions are essential for con-
jugation, mating occurs only under certain environmental
conditions and the environment modifies the time of onset,
the intensity and the duration of the epidemic of conjuga-
tion. The factors which have most often been considered
as important are food and its correlatives, temperature,
salts (type and concentration), pH, metabolites and popu-
lation density.

Food has been considered the most important single
factor. In mass cultures conjugation occurs when con-
ditions have become somewhat adverse for multiplication.
Maupas (1889) surmised that lack of food was a necessary
condition for conjugation. Jennings (1910) reported that
it was rather the lack of food after a period of plenty.
Many other conditions are changing along with the change
in food, and Bělař (1924), using *Actinophrys sol,* offered
the first conclusive proof that decline in food after a period
of plenty was indeed the most important factor. Similar
experiments with the same results have been performed
here with a number of stocks of *P. multimicronucleatum*
and *P. caudatum* (Giese, 1935, 1938a, and Giese and
Arkoosh, 1939).[3]

[3] Baitsell (1912) observed that conjugation occurred in some substrates,
not in others. In experiments carried out here on *P. multimicronucleatum*
(stock 1 was used) substrate was found to be of no consequence except that
in more nutritious substrate conjugation was proportionally delayed, but
equally good epidemics occurred in lettuce, hay, wheat, mushroom, apple and
rice infusions.

Temperature affects the time of onset, the intensity and the duration of an epidemic of conjugation. Conjugation occurred between 8.4° and 30° C., but the number in union at these extremes was very small. Animals grown and kept at 15°, 20° and 26° C. will conjugate when the food is depleted, the conjugants appearing soonest at the highest temperature. A temperature change is thus not necessary for conjugation, yet in most cases a change from 26° C. to 20° C. was followed by an epidemic of greater intensity (65 per cent.). This was true especially for animals of stocks I and II which showed relatively few conjugants when grown and kept at 26° C. Placed at 15° C., however, the number of conjugants was smaller than at 20° C. Stocks 2E, 6, 7 and 9 showed good epidemics (up to 60 per cent.) even when grown and kept at 26° C. Animals of all stocks grown and kept at 30° C. seldom conjugate, but when they are placed at lower temperatures at the appropriate time, conjugation epidemics occur.

When animals which were showing the mating reactions at 20° C. were subjected to 8.4° C., they disbanded almost completely, union being rare. When such a culture was returned to 20° or 26° C., the mating reactions began again even when twelve or more hours intervened. In some instances this was repeated a second time. The reacting state is apparently maintained for a long time at this lower temperature, but the reactions can not be completed until the temperature rises to a suitable level. Subjection of cultures to 5.4° C. acted similarly when it was not lethal. When animals in the reacting state are subjected to 30° C., they rapidly lost the tendency, indicating a reaction with a fairly high temperature coefficient.

To determine the effect of different temperatures upon duration of epidemics of conjugation, conjugants of 2E were picked from a culture when a maximum were in conjugation and were suspended in culture fluid at 15, 20 and 26° C. The results, plotted in Fig. 1, show that the duration of the epidemic is directly dependent upon the temperature.

Salts of a specific type and in certain ranges of concentration have been considered as necessary for conjugation by Enriques (1909) and Zweibaum (1912). With stocks of *P. multimicronucleatum* used here this is apparently not the case. It is true that paramecia washed and suspended in distilled water conjugated but rarely and that too high
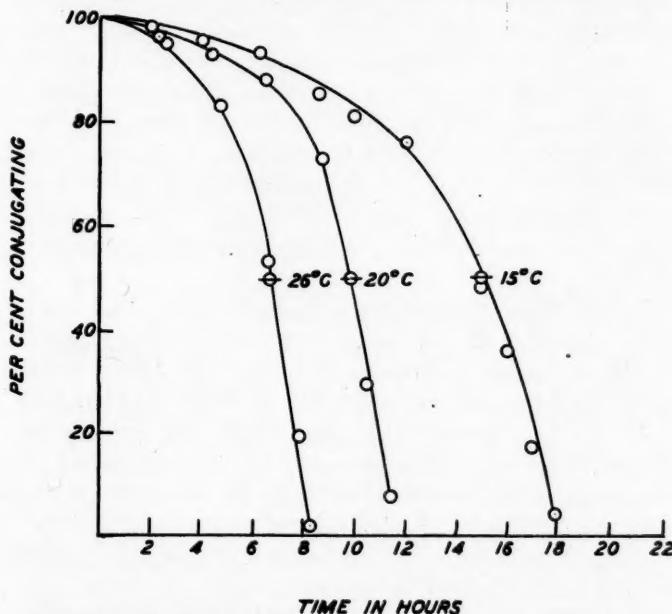
FIG. 1. Rate of ex-conjugation at different temperatures. Conjugants (stock 2E) were removed when the epidemic was at a maximum. Each curve is the average for 800 animals.

a concentration (0.2 per cent. balanced salt solution)[4] also prevents conjugation. However, the culture medium (such as lettuce or hay infusion) can be replaced by balanced salt solutions of a considerable range of concentration (0.15 to 0.003 per cent.) without preventing epidemics of conjugation. Furthermore, addition of $FeCl_3$ or $AlCl_3$,

[4] See Giese, 1938a, for a description of the balanced salt solution used here. A 0.3 per cent. (total salt) solution of this medium was lethal to the paramecia (stock 1).

claimed to be so effective by Zweibaum for increasing intensity of conjugation epidemics in *P. caudatum,* had no significant effect on *P. multimicronucleatum.* Thus cultures in balanced salt solutions buffered at pH 7.0 made up to 0.0000166M. $FeCl_3$, showed 69.0 per cent. (with a standard deviation of ± 9.1 per cent.) in conjugation, the controls, 60.0 ± 7.5 per cent. Other cultures suspended in $AlCl_3$ solutions of six different concentrations (between 0.000416M and 0.00000208M made up in balanced medium buffered at pH 7.0) showed epidemics about equivalent to controls and, in all, averaged 62.1 ± 5.8 per cent., the controls, 61.0 ± 12.3 per cent. And finally, suspension in pure salt solutions[5] of NaCl, $MgSO_4$ and $CaCl_2$ (each 0.012 per cent.) reduced the intensity of the epidemic to between 20 and 40 per cent. of the population but did not prevent conjugation.

To determine if the action of the salts in the medium is mainly osmotic, the paramecia were washed and suspended in 0.007M sucrose (approximately equivalent to the ionic molarity of the balanced salt solution buffered at pH 7.0)[6] In all cases paramecia conjugated comparably to controls. Whatever the other functions may be, the function of the salts is apparently mainly to maintain the osmotic relations, since most of the salt can be replaced by sucrose.

That the duration of an epidemic of conjugation is also not greatly influenced by the salt content of the medium is indicated by experiments summarized in Fig. 2. Exconjugation occurs at about the same rate in buffered and unbuffered salt solutions, in sucrose solution and in culture medium.

A change in *hydrogen ion concentration* affects many biological processes, therefore tests were made to determine what effect a variation in pH had upon conjugation.

[5] Dilution of original medium 80,000; 60,000; and 10,000 fold in the three cases, respectively.

[6] Dilution of salt medium 1,500–1,800 fold in these experiments. Similar experiments on paramecia with 0.012 per cent. urea gave similar results. Experiments with *Blepharisma undulans,* using sucrose, also gave similar results.

Little difference was observed in the intensity of conjugation for the pH range studied, those at pH 6.0 showed 58.1 ± 6.7 per cent. in conjugation; at 7.0, 59.0 ± 8.6 per cent.; at 8.0, 65.5 ± 6.0 per cent.[7] In Fig. 2 it is observed that this pH range also has but little effect upon the duration of conjugation.
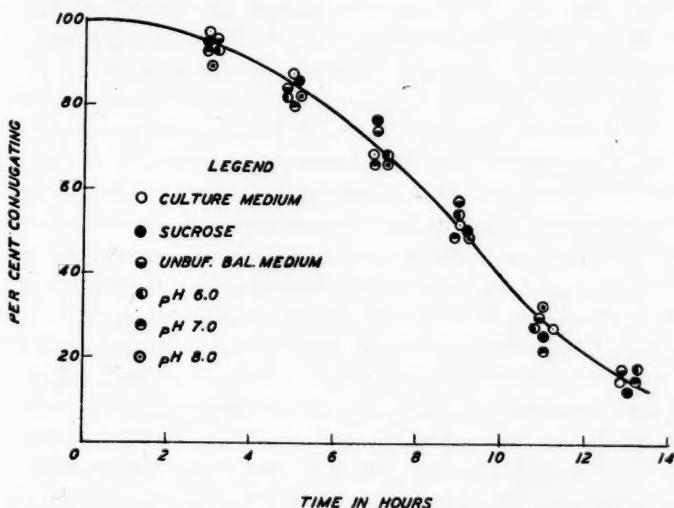
FIG. 2. Rate of ex-conjugation at 20° C. of conjugants (stock 7) subjected to various solutions when at the height of an epidemic. The curve is the average for 300 animals in each solution.

Crowding a flourishing culture is a favorite way of producing an epidemic of conjugation. Crowding might act in one of several ways: by (1) increasing the concentration of metabolites; (2) increasing the chance for contact between individuals; (3) giving a greater chance for selection of suitable mates; (4) bringing about a quicker exhaustion of the food.

When animals are washed free of food in salt solutions and one group is concentrated twenty times as much as the other, conjugation occurs at approximately the same time

[7] Solutions and buffers made up as described in Giese, 1938a. Stocks 1, 6 and 7 tested.

in the two cultures. But if both are fed a given quantity of bacterial suspension, conjugation occurs much sooner in the concentrated culture, the time difference depending on the quantity of bacteria added. There is thus no correlation with metabolites which are more concentrated where the paramecia are more numerous but a direct correlation with exhaustion of food.[8]

To test the third possibility—whether selection of mates was facilitated by larger numbers—incipient mating pairs of stock 7 were isolated in hanging drops in moist chambers and in only 21 per cent. of the drops did conjugation occur. When four paramecia were present in a drop, conjugation occurred in 49 per cent. of the cases. When 12 paramecia were present in a drop, conjugants appeared in every drop. In a series in which an average of 44 animals were present per drop, 39.4 per cent. of the total population conjugated (in some extreme cases 84 per cent. of the population of a given drop conjugated.[9] In all experiments contact was readily established and the pairs swam together for some time before separating. It is readily seen from the above that contact is not lacking, yet some animals seem to find their mates unsuitable. It is known that large animals tend to mate with large, small with small (Jennings, 1911), although there are exceptions to the rule (Giese, 1938b). Other factors at present less obvious than size may play a part in selection as well.

DISCUSSION

Experiments have demonstrated mating types stable for a given caryonide in only one of eight local stocks of *P.*

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[8] Metabolites of various bacteria also seem neither to accelerate nor to retard conjugation. Paramecia (stock 1) suspended in Seitz-filtered infusions of *Pseudomonas fluorescens, Bacillus subtilis, B. prodigiosus, B. pyosepticus* and various mixed cultures conjugated as well as controls.

[9] Even more striking results were obtained with a stock (No. 12) of *P. caudatum* showing clear-cut mating types. When two individuals were present, of 151 cases, 35.8 per cent. conjugated; when four paramecia were in a drop, conjugants appeared in 71.5 per cent. of the drops (35 cases); when six paramecia were in a drop (5 of one mating type, one of the other), conjugation occurred in 85.5 per cent. of the drops (68 cases).

multimicronucleatum. That this finding is not peculiar to
the local stocks only is indicated by the fact that Gilman
(1938) has made similar observations on stocks of this spe-
cies collected in the vicinity of Johns Hopkins University.
It is not impossible that in *P. multimicronucleatum* the evo-
lution of such mating types is only beginning. More exten-
sive experimental data are, however, necessary to show
whether this is the case or whether evolution has gone on
in a direction quite different from that in *P. aurelia* or
P. bursaria.

Both internal and external factors influence the conjuga-
tion of *P. multimicronucleatum.* Thus after conjugation
paramecia of some of the stocks must achieve an internal
state of "maturity" before another conjugation. Also
paramecia of race 2C will not conjugate in the absence of
2E; other stocks appear to be self-fertile. But in addition
to the satisfaction of the above internal conditions, the
animals must be subjected to certain external influences
before conjugation will occur.

While many environmental factors modify the onset,
intensity and duration of an epidemic of conjugation, in
many cases the action is mainly if not entirely by their
effect upon the food available and the nutritive state of the
protozoans. Thus if paramecia are grown at different
temperatures they conjugate soonest at the highest tem-
perature, but in each case when the food supply is begin-
ning to be depleted. Also washing the paramecia free of
food in appropriate salt solutions facilitates conjugation.
And crowding paramecia in ordinary infusions favors con-
jugation in part by bringing about a quicker exhaustion of
food. Depletion of the food leads to the development of
an internal nutritive state conducive to conjugation.

This nutritive state favorable to conjugation varies in
different species. Thus Sonneborn (1938) observed that
in certain stocks of *P. aurelia* conjugants appear even
while the animals are still very vigorous and even contain
food vacuoles. Jennings (1938) found that a certain
amount of starvation was necessary for *P. bursaria.* Zwei-

baum (1912) reported that considerable starvation was necessary for *P. caudatum*. Of the stocks of *P. multimicro-nucleatum* used here, stocks 1 and 11 required more starvation than the others, and even when mating types were present as in stock 2, a certain degree of starvation was also found to be essential, as discussed elsewhere (Giese and Arkoosh, 1939).
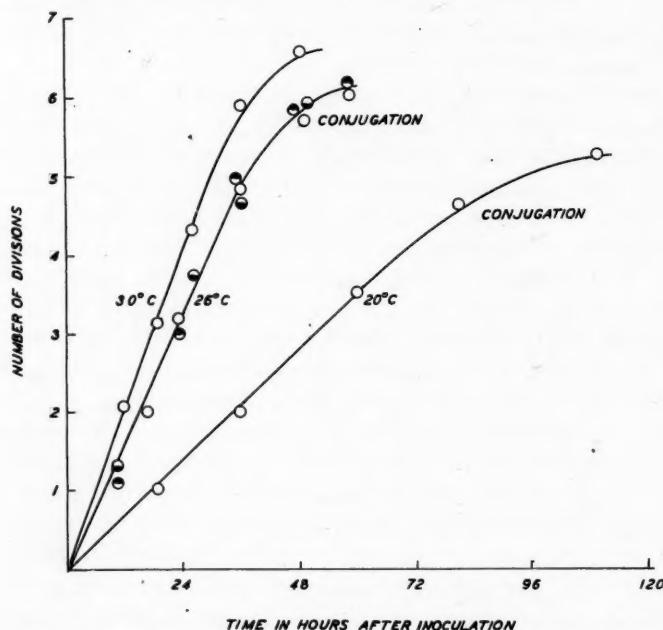
Fig. 3. Appearance of conjugants at different temperatures. Each point is the average of 10 cultures (stock 7).

While the exact state favorable to conjugation varies for different stocks of a species and for different species, in all cases there is one common denominator—a change from the active growth and multiplication characteristic of the vegetative state to a state of slower growth and multiplication. For animals ready to conjugate are growing less actively, have become reduced in size (Jennings, 1911) and in some cases are consuming less oxygen (Zweibaum,

1921). Conjugants are observed in a population only after an inflection in the growth rate curve (Fig. 3), or in the extreme cases after the division rate is zero.

That it is the relative change in division and growth rate, not an absolute change, is shown by the fact that the absolute rate of multiplication is different at 20, 26 and 30° C., yet as illustrated in Fig. 3, in each case conjugation follows a decrease from the division rate characteristic of each temperature (at 30° C. conjugation occurs only if the paramecia are placed at the proper time at a lower temperature). Conjugants are smaller than non-conjugants or vegetative individuals. The size reached before conjugation occurs, *i.e.*, the decline in nutritive state, preceding conjugation is also relative, as was demonstrated for *Blepharisma* (Giese, 1938b) and depends upon the preceding nutritional history of the animal. The relative change in nutrition seems to set going a chain of reactions which lead to the "sticky state" characteristic of the animals in incipient conjugation.

That the changes in appearance, activities and nutrition preceding conjugation are superficial outward signs of a more fundamental change in internal state is granted, and it is the search for the nature of this fundamental change that invites one to further research.

SUMMARY

(1) Experiments on *Paramecium multimicronucleatum* indicate that mating types occur, although most of the stocks examined exhibit conjugation among progeny of a single individual.

(2) Environmental factors modify the time of onset, the intensity and duration of an epidemic of conjugation, and, when variation from the usual condition is extreme, may prevent conjugation.

(3) Some environmental factors, if varied within relatively small limits, have very little influence on the epidemic of conjugation, *e.g.*, pH, salts and metabolites.

(4) Other factors of the environment, such as tempera-

ture and crowding of the paramecia, may modify the time of onset, the intensity and duration of an epidemic of conjugation, partly by direct effects, partly by effects upon the rate of exhaustion of the food.

(5) A relative decline in nutrition precedes conjugation in all stocks, whether they show selfing or mating types.

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MATING TYPES IN *PARAMECIUM CAUDATUM*[1]

LAUREN C. GILMAN

DEPARTMENT OF ZOOLOGY, JOHNS HOPKINS UNIVERSITY

THE following account of mating types in *Paramecium caudatum* is a preliminary report of investigations on the number and interrelations of the types, on the conditions required for the mating reaction and conjugation, and on the inheritance of type within the clone.

The material examined consists of cultures derived from individuals collected from ten different natural habitats: seven in or near Baltimore; two near Woods Hole, Mass.; and one in Connecticut. From each collection one to six animals were isolated and cultures grown from each. In addition, in those collections in which conjugation took place, pairs that had not yet firmly united were separated and cultures grown from each member of the pair. Since presumably the animals starting to conjugate were of different mating types the cultivation of split pairs greatly increased the chances of obtaining cultures of opposite mating type from the same natural source.

In order to discover whether mating types occurred among these clones and, if so, how many there were and how they were interrelated, samples from each clone were mixed, in twos, with samples from each of the other clones and the mixtures were examined for conjugants. When only small cultures were available about fifty animals from each culture to be tested were used in each test. Since in these small cultures the animals were not, as a rule, in the proper condition to give an immediate reaction they were placed in four drops of culture fluid in order that they might be in a position to conjugate when they reached the proper condition. The mixtures were placed at 24° C.

and examined for conjugants after twenty-four hours.   As
controls, one hundred animals of each clone were kept un-
mixed but otherwise treated in the same manner as the
mixtures.   When conjugation occurred in the mixture but
not in the controls the two cultures mixed were regarded
as being of opposite mating types.   If larger cultures, in
which the animals were in reactive condition and aggre-
gated in a dense band on the side or bottom of the con-
tainer, were available, larger numbers were mixed and the
mixtures examined immediately for the characteristic
rating reaction, clumping of the paramecia.

The results of these clonal mixtures are given in Table 1

TABLE 1

INTERACTION OF CLONES OF PARAMECIUM CAUDATUM ISOLATED FROM NATURAL COL-
LECTIONS.   THE LETTER GIVES THE SOURCE OF THE CULTURE, THE ROMAN
NUMERALS THE MATING TYPE.   CONJUGATION IS INDICATED BY +,
LACK OF CONJUGATION BY −

	CI	DI	CII	DII	MIII	PIII	WIII	EIV	MIV	UIV	WIV
CI	−	−	+	+	−	−	−	−	−	−	−
DI	−	−	+	+	−		−				−
CII	+	+	−	−	−		−			−	−
DII	+	+	−	−	−		−			−	−
MIII	−	−	−	−	−	−	−	+	+	+	+
PIII	−		−	−	−	−	−	+		+	+
WIII	−	−	−	−	−	−	−	+	+	+	+
EIV	−				+	+	+	−	−	−	−
MIV	−				+		+	−	−	−	−
UIV	−		−	−	+	+	+	−	−	−	−
WIV	−	−	−	−	+	+	+	−	−	−	−

where + signifies that conjugation occurred in the mixture
of the clones represented on the corresponding row and
file, and − signifies that it did not.   A blank signifies that
the mixture was not tried.   Clones collected from the same
source in nature are represented by the same letter.   As
shown in the table the clones in collections C and D form
one interbreeding group, those in collections E, M, P, U
and W form another; the two groups are designated 1 and
2, respectively.   In each group there are but two mating
types; the two in Group 1 are designated I and II, those
in Group 2, III and IV.   In each of the collections C and D
occur some clones of type I and some of type II; only one

of each type is shown in the table. Similarly, in each of the collections W and M occur some clones of type III and some of type IV; but collection P contained only clones of type III, and collections E and U contained only clones of type IV. The clones in the remaining collections (F, G and Q) have not yet been examined sufficiently to be placed with respect to the other collections.

The system of mating types and groups shown in Table 1 resembles that reported for *P. aurelia* by Sonneborn (1937, 1938) in that each group consists of but two interbreeding mating types; and differs from the system reported for *P. bursaria* by Jennings (1938a, 1938b) where four or more interbreeding types are found in each group.

Conditions for Conjugation and the Mating Reaction

In both Group 1 (types I and II) and Group 2 (types III and IV) tests were made concerning the relation of the mating reaction to the time of day. In Group 2, mixtures were made at all times of the day and night at hourly intervals and conjugation appeared to occur with equal readiness at all times. In Group 1 hourly tests were made only for the period from 11 P.M. to 6 A.M. Conjugation occurred at all times during this period. However, in mass cultures of this group the characteristic clumping reaction has been observed to occur at all hours of the day. This disagrees with the observations of a number of earlier investigators of *P. caudatum,* who found this species conjugates usually about 4 A.M. Perhaps certain groups show a diurnal periodicity while others do not, as in *P. aurelia* (Sonneborn, 1938) and *P. bursaria* (Jennings, 1939).

The effect of diverse temperatures upon the number of conjugants formed in mixtures of types I and II has been investigated. Since it has been observed that the addition of new culture fluid inhibits conjugation for a time the mixtures of types I and II were placed in four drops of culture fluid before placing at the various temperatures in order that conjugation would not occur before the cultures had come to the temperature being tested. The mixtures

were examined at twelve-hour intervals for conjugants, and all pairs which had formed were counted and removed with a pipette.   No conjugation occurred at 9°, 30° or 34° C.   The best results were obtained at 18° and 24° C., but it took several days longer to obtain the maximum number of conjugant pairs at 18° than at 24° C.   At 27° C. conjugation occurs, but fewer pairs are formed than at 18° or 24° C.

TABLE 2

INTERACTION OF SPLIT PAIRS OF CLONE M, TYPE III, GROUP 2 OF PARAMECIUM CAUDATUM.   SP3a REPRESENTS MEMBER a OF SPLIT PAIR 3

	WIII	MSP3b	MSP6a	MSP8b	MSP9a	MSP3a	MSP6b	MSP8a	MSP9b	WIV
WIII	−	−	−	−	−	+	+	+	+	+
MSP3b	−	−	−	−	−	+	+	+	+	+
MSP6a	−	−	−	−	−	+	+	+	+	+
MSP8b	−	−	−	−	−	+	+	+	+	+
MSP9a	−	−	−	−	−	+	+	+	+	+
MSP3a	+	+	+	+	+	−	−	−	−	−
MSP6b	+	+	+	+	+	−	−	−	−	−
MSP8a	+	+	+	+	+	−	−	−	−	−
MSP9b	+	+	+	+	+	−	−	−	−	−
WIV	+	+	+	+	+	−	−	−	−	−

In Group 2 (types III and IV) the effect of temperature has not yet been investigated, but observations of considerable interest have been made on the effect of diverse nutritive conditions.   The various stages of nutritive decline from the well-fed to the starved condition may be represented by the numbers 1 to 5, respectively.   When cultures of types III and IV in these diverse nutritive states are mixed, the following behavior is observed:

(1) Animals very well fed and plump.   No mating reaction or conjugation.

(2) Animals well fed but not plump.   A weak mating reaction; a few pairs cling together but break apart without conjugating.

(3) Animals of moderate size, not well fed.   Strong mating reaction: many clumps form; these disintegrate into pairs which fuse and complete conjugation.

(4) Animals small and thin.   Strong mating reaction:

many clumps form; these disintegrate, but few or none of
the animals proceed to conjugate.

(5) Animals very small and starved. No mating reac-
tion and no conjugation.

Inheritance of Mating Type

All the clones reproduced true to type for periods of at
least two weeks, and all but two for a period of at least one
month. Hence the mating types are strictly inherited dur-
ing vegetative reproduction for considerable periods of
time. However, in most of the clones conjugation eventu-
ally occurs. In order to ascertain whether such conjuga-
tion is the result of the presence in the culture of animals
of diverse mating types, pairs not yet firmly united were
separated and each of the two individuals separately cul-
tured. Table 2 gives the results of tests for mating types
made on four such split pairs removed from a culture of
type III of clone M, Group 2. As appears in the table the
culture from one member of each split pair conjugated with
type III, not with type IV, while the culture from the other
member of each split pair conjugated with type IV, not
with type III. This shows, therefore, that when conjuga-
tion occurs in a clone that has long produced individuals
of one type only, it is the result of the production of both
mating types within the culture. When individuals of
each type are isolated from such a culture, each again re-
produces true to type for long periods: a month in the case
of the one that was the same type as the original culture,
i.e., type III, and at least three months in the case of the
new type, IV.

The fact that the type IV cultures from such split pairs
remained true to type longer than the type III cultures
agrees with observations on the clones isolated from nat-
ural collections. Some of the original clones of type IV
have continued to reproduce true to type for the entire
period of nine or ten months that they have been under
observation. This is, however, not true for all clones of
type IV, nor is it true for any clone of type III. In Group

1 a somewhat similar condition has been observed in clones isolated from nature. Of five clones of type I, four have remained true to type for a period of nine to ten months. The fifth clone produced a few pairs of conjugants about five months after it was isolated from the wild. Two of the clones of type II which have been isolated from nature give epidemics of conjugation without mixture every two to three weeks.

These genetic relations are similar to those found in *P. aurelia* by Sonneborn (1937; 1938; 1939) and by Kimball (1937): some stocks give rise to both mating types characteristic of·their group; others remain permanently of one type. In *P. aurelia* the changes of type within a clone are consequences of nuclear reorganization (endomixis or autogamy). It has also been shown by Giese and Arkoosh (1939) that in one of the mating types of *P. caudatum* studied by them conjugation within the clone occurs only after endomixis; presumably this is the basis of the changes in the present clones of *P. caudatum,* but this matter has not yet been investigated.

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MATING TYPES IN EUPLOTES[1]

RICHARD F. KIMBALL
STERLING RESEARCH FELLOW
OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

FROM the previous papers of this symposium, it can be seen that much information has been gained concerning mating and, in particular, mating types and their inheritance in the genus Paramecium. It seemed desirable to extend this type of investigation to another ciliate genus not closely related to Paramecium. For this purpose, a study was undertaken of the hypotrichous ciliate, Euplotes. The work on this organism is still in the early stages, but it is already possible to say that mating types similar to those found in Paramecium are found in it also.

The processes occurring during the life of this organism are slightly different from those found in Paramecium. The only difference in the process of conjugation that is of any importance for present purposes is that only one macronuclear anlage is formed in each ex-conjugant instead of two or more. Therefore, the terms clone and caryonide in this organism refer to the same unit. No encystment or endomixis has been seen, though careful watch was kept for both. The reports of endomixis-like processes in Euplotes that have appeared in the literature to date do not indicate that any replacement of the macronucleus from the micronucleus occurs. The formation of new caryonides at endomixis, therefore, apparently does not occur in Euplotes.

To turn to the results of the investigations of mating, when animals of certain clones are mixed, large numbers of conjugant pairs are formed in the mixture. However, in no case, has any immediate clumping together of large

numbers of animals, such as is so characteristic of the mating reaction in Paramecium, been observed. In fact, no sticking together of any sort is seen until at least an hour and a half and frequently longer after mixture. After this time, the animals become comparatively quiet and more or less aggregate in the bottom of the depression of the slide in which they were mixed. In appropriate mixtures, pairs of animals then begin to stick together. During this process, there was never observed what could be considered a clear group of more than two animals attached to each other. This process of gradual sticking together of two animals is very different from the sudden sticking together of large numbers of animals that occurs in the mating reaction in Paramecium.

The conditions under which conjugation will occur when appropriate clones are mixed are somewhat like those found for certain races of Paramecium. The animals mate best when they have been well fed and have almost exhausted the food supply. In practice, it has been found best to add a small amount of food to the mixture when it is made. This food supply is exhausted within a few hours; and, if a suitable mixture was made, the animals start to mate. There was no indication of any effect on mating of the time of day or of temperature within a range of 18° to 31° C. As in some stocks of Paramecium, animals that have recently conjugated will not conjugate again until a certain period has elapsed; in Euplotes, a period of a month or more.

I will now turn to the results of mixing various clones. In the first place, all the clones studied can be divided into five groups. No mating has been found in mixtures of any clones of different groups. Appropriate mixtures of clones of the same group do, however, contain conjugants. Between some of these groups, there are considerable morphological differences, while between others the differences are slight or non-existent. Though they are all closely related to *Euplotes patella,* no classification of Euplotes proposed up to now fits all these groups. I do not at

present wish to go into the taxonomy of Euplotes nor to set up species until more material has been studied. I merely wish to point out that the categories that have for present purposes been called groups differ from the groups found in Paramecium in being more or less morphologically dissimilar from each other.

Only one of these groups has been analyzed in detail for mating types. In this group, 55 clones have been studied. While all possible mixtures between these clones have not been made, a considerable number have; and from the results of these mixtures it is possible to divide the clones of this group into six mating types. Conjugation occurs in mixtures of clones of different mating type but not in those of the same mating type. As an example, the interactions among 20 clones between which all possible mixtures were made are shown in Table 1. The situation in this group, then, is very much like that found in *Paramecium bursaria,* in which, unlike *Paramecium aurelia,* more than two mating types occur in each group.

The inheritance of the mating types at conjugation has not been fully worked out. However, it is known that, as in Paramecium, change of mating types occurs at conjugation. As in *Paramecium bursaria,* clones of mating type diverse from that of either parent are produced as well as those like the parents.

As in Paramecium, mating was found in some cases within unmixed samples of one clone. That this mating is not, in all cases at least, due to change of mating type following replacement of the macronucleus at some endomixis-like process is shown by one experiment. Two animals were isolated and each allowed to give rise to a small mass culture. These cultures were examined regularly, and samples were stained every day. No signs of any unusual nuclear changes were observed. Since in Euplotes new macronuclear anlagen are clearly visible with low magnifications in the living animal, any replacement of the macronucleus by the formation of a new anlage would have been seen. None was. Nevertheless, conjugation

TABLE 1

THE INTERACTIONS BETWEEN 20 CLONES OF SIX MATING TYPES, ALL BELONGING TO
ONE GROUP.   THE PLUS SIGN INDICATES THAT CONJUGATION OCCURRED
IN THE MIXTURE IN QUESTION ; THE MINUS SIGN, THAT IT
DID NOT.   ALL THE CLONES OF A MATING TYPE
ARE GROUPED TOGETHER

	52b	53b	67a	57b	40b	41b	49b	56b	21a	44b	45b	58b	60b	43b	46b	63b	68b	69a	73a	69b
52b	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
53b	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
67a	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57b	−	−	−−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40b	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
41b	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
49b	+	+	+	+*	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
56b	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
21a	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
44b	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+
45b	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+
58b	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+
60b	+	+	+	+	+	+	+	+'	+	−	−	−	−	+	+	+	+	+	+	+
43b	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+
46b	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+
63b	+	+	+	+	+	+	+	+	+	+.	+	+	+	−	−	−	−	+	+	+
68b	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+
69a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
73a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
69b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−

occurred within these two cultures.   It must be concluded
that the vegetative progeny of a single individual can mate
with each other, in some cases, though no replacement of
the macronucleus has taken place.   Whether this involves
a change of mating type during vegetative reproduction
such as has been found in *Paramecium aurelia* has not yet
been determined.

In Euplotes, it has been possible to show that in many
mixtures in which mating occurs this mating is not only
between animals of different clones but also between those
of the same clone.   This unexpected relation was demon-
strated by the use of double animals.   In order to make the

following discussion clear, a brief description of these animals will be given.

In protozoan literature, there have been reports from time to time of the occurrence of abnormal animals composed of two individuals fused together in some manner. Some of these double animals have been so constructed that when they divided two double animals like the parent were produced. Such "stable" double animals can, then, give rise to a clone all the animals of which are double. The double Euplotes that was used in this work was of this sort. It is composed of two animals fused by their right aboral surfaces and is homopolar. The two fused animals instead of remaining more or less flat like normal single animals are bent through a considerable angle along the line of fusion.

The oral surfaces of the two halves of a double are free; and, therefore, it is physically possible for the doubles to conjugate with other animals. Actually in appropriate mixtures with single animals, conjugation between the single and the double animals does take place. However, in these same mixtures very frequently conjugant pairs composed of two single and more rarely of two double animals occur. This means, then, that animals of the same clone are mating with each other. Nevertheless, unmixed samples of these clones, set aside at the same time that the mixtures were made, contained no conjugants. Thus mixture of one clone with another appears to induce conjugation between animals that otherwise would not have conjugated with each other.

Further light on the way in which this induction was brought about was obtained by mixing animals from one clone with culture fluid (without animals) taken from a culture of another clone. This culture fluid was pipetted off from the culture and examined carefully for animals. All that were found were removed. Animals of the clone to be tested were then added to it. In some cases, considerable conjugation occurred among these animals, though none was found among animals from the same clone un-

mixed with foreign culture fluid.   Culture fluid from clones of single as well as double animals had this effect.   It must be concluded that certain clones, perhaps all, have the ability to so change the fluid in which they are growing that it will induce conjugation among animals of certain other clones.

Sonneborn has found in *Paramecium aurelia* that culture fluid from one caryonide does not induce conjugation among animals of another.   However, he finds that contact with an animal of opposite type makes an individual capable of forming temporary pairs with animals of its own type.   These pairs do not last long, however, so that final conjugation occurs only between animals of opposite type.   In Euplotes, on the other hand, pairs formed between animals of the same clone regularly complete conjugation.

What the relations are between the induced mating within a single clone of Euplotes and the mating types is not certain.   It is clear, of course, that two clones of the same mating type do not induce mating within each other since when they are mixed no conjugation occurs.   In many mixtures of clones of double and single animals, conjugation takes place between the doubles and the singles; and in a few mixtures this is the only sort of mating that has been seen.   Therefore, not all mating within mixtures is mating within a clone induced by the presence of animals or fluid from another clone.   However, it is too early to say whether there are any other systematic relations between induced mating and the mating types.

THE VISIBLE ORGANIZATION OF THE GIANT SALIVARY GLAND CHROMOSOMES OF DIPTERA

DR. C. W. METZ

DEPARTMENT OF EMBRYOLOGY, CARNEGIE INSTITUTION OF WASHINGTON, BALTIMORE, MD.

IT is well known that ordinary chromosomes of animals and plants, when in the condensed mitotic stages, contain coiled chromonemata, often clearly visible under the microscope. Such chromonemata were identified as early as 1908–1911 by Bonnevie (1908) and by Vejdovsky, and were familiar to Alverdes (1912) when, in his studies on dipteran salivary gland nuclei, he described the organization of the giant "spireme," which is now known to represent giant chromosomes. Alverdes naturally attempted to interpret the structure of these giant bodies in terms of the chromonemata found in "ordinary" chromosomes. But in doing this he was led to a conception which has subsequently had to be abandoned. Since the view was quite in keeping with conceptions then current, however, it was readily accepted and was supported for a long time by other observers, thus serving to delay recognition of the true significance of these structures for twenty years.

More recently other observers, although not accepting the particular view of Alverdes, have likewise attempted to interpret the organization of these giant chromosomes in terms of *visible* chromonemata like those seen in "ordinary" chromosomes. And from their accounts it is difficult to avoid the conclusion that these authors have similarly been led to their views largely through the influence of what is seen in "ordinary" chromosomes. Such seems to be the case not only as regards the interpretation of Koltzoff (1934) and of Bridges (1935), who described "chromonemata" coiled around a central axis in these chromosomes (a view now abandoned) (Bridges, *a*) but also as regards that of Koller (1935), Bauer (1936) and Painter and Griffen (1937), who described "chromone-

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mata" here in larger numbers, extending diagonally or straight along the chromosome, and filling it uniformly. Although it is, of course, important to determine the relationship between the constituents of the giant chromosomes and those of other chromosomes, it is not necessarily true that these constituents will show the same morphological characteristics in the two cases. Indeed, there is strong evidence that they do not.

As indicated in earlier papers (Metz, 1935, 1937; Metz and Lawrence, 1937), our evidence in this laboratory indicates that the giant chromosomes present a distinctly different problem from "ordinary" chromosomes, as regards structure, and that the pattern discernible in the former is not made up of visible chromonemata. The problem has been treated in the papers just referred to (Metz, 1935, 1937; Metz and Lawrence, 1937); but since there still seems to be some confusion as to the nature of the evidence and of the conflicting interpretations, a brief discussion of certain points is presented here. The discussion concerns especially (1) comparison of the giant chromosomes with "ordinary" chromosomes, and (2) comparison of current views as to the structure of the former. Since Koller (personal communication) has abandoned his earlier view and adopted that of Metz and Lawrence, the latter comparison involves three interpretations, that of Bauer, that of Painter and Griffen and that of Metz and Lawrence.

Since different investigators of salivary gland chromosomes have used different genera of flies, the writer has made a careful study of conditions in the four genera used (Chironomus, Drosophila, Sciara, Simulium) to make sure that, as is actually found to be the case, the structure is fundamentally the same in all.

A salivary gland chromosome represents a pair of homologous chromosomes fused side by side to form an essentially cylindrical body which, in the larger nuclei, is approximately a hundred times as long as, and thousands of times the volume of, the comparable chromosomes in "ordinary" somatic cells at metaphase. The chromosomes are coiled about in the nucleus, and, unlike those of ordi-

nary cells, they occupy practically the entire volume of the nucleus (Doyle and Metz, 1935; Buck and Boche, 1938). The latter fact is especially significant in the present connection, for, together with other evidence, it shows that most of the nucleoplasm is here contained *within* the chromosomes, making the morphological problem presented by these chromosomes distinctly different from that presented by "ordinary" chromosomes in cells capable of mitosis.

The primary pattern, visible under the microscope in the salivary gland chromosome, is made up of transverse chromatic disks or "cross-bands," and in a gross sense is constant for each chromosome, the disks presumably reflecting in some manner the linear seriation and localization of the genes. Present interest centers partly in the nature of these disks, but more especially in that of the faint longitudinal lines which extend from one disk to the next across the intervening achromatic zones (*D* and *E,* in Fig. 1).

The salivary gland chromosome is commonly considered as a multiple structure, owing its size to repeated divisions of the chromonemata present before the enlargement began. There are good theoretical grounds for this view, although no demonstrative evidence has yet been secured. On the basis of size, either of chromosomes or of nuclei, however, the number of chromonemata here should be a thousand or more, rather than the small numbers described on the basis of visible constituents. According to Bauer the faint longitudinal lines just referred to are true threads and represent true chromonemata. According to the revised view of Painter and Griffen (1938) they are true threads, but represent bundles of chromonemata in which the individual chromonemata are sub-microscopic and hence invisible. On these two views the granules or chromatic droplets represent either individual chromomeres (Bauer) or bundles of sub-microscopic sister chromomeres (Painter and Griffen), aligned on the chromonemata.

The transverse chromatic disks, often granular in appearance, together with the delicate longitudinal lines,

make up a visible pattern which varies under different conditions according to the distribution of the chromatic material and, presumably, degree of internal pressure. Sometimes the disks appear as relatively sharp, straight cross-bands, and the longitudinal lines are faint (Fig. 2, A). In other cases the disks appear as zigzag bands and the longitudinal lines are prominent, as represented sche-
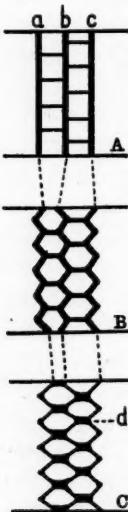
Fig. 2. Diagram illustrating how the appearance of one region may vary with differences in the distribution of chromatin and, presumably, differences in internal pressure. In *A* the disks (*a*, *b*, *c*) are essentially straight and contain most of the chromatin. (Actually, of course, they are not as smooth in outline as shown here.) In *B* the disks are zigzag in outline and the achromatic droplets hexagonal in optical section (as in Fig. 1 and in Fig. 3 at *c*). In *C* the chromatin, instead of being mainly in the disks, lies mainly in the spaces between the achromatic droplets separating the disks. Here it forms conspicuous granules, each of which contains material from two disks (compare region *d* in photograph in Fig. 3). These conditions are not hypothetical, but are actually observed.

matically in Fig. 1 and Fig. 2, B. Between these two extremes is a complete range of intermediate conditions.

According to the interpretation of Metz and Lawrence (1937) the giant chromosome, at least in the fixed condition, is made up of a series of chromatic disks which,

although connected by true chromosome material, are separated by layers of achromatic droplets presumably representing the nucleoplasm or "matrix" material. Each two successive disks are separated by one layer of droplets embedded in the true chromosome substance, which extends longitudinally between and around the droplets from the one disk to the next, as indicated in Fig. 1. Ordinarily, of course, only a few adjacent droplets are seen in median optical section at one focal level, as seen at *c* in Fig. 3, instead of a large number as shown in the diagram. Likewise, the number of droplets, like that of the granules, actually differs widely at different loci along the chromosome instead of being uniform as shown here. Otherwise, however, the diagram is essentially accurate.

On this view the delicate longitudinal lines (*D, E,* Fig. 1) are not threads, as considered by Bauer, Painter and Griffen and earlier authors, but are optical sections of the material lying between the droplets. This material may, of course, be made up of a multitude of sub-microscopic chromonemata tightly packed together; but the visible image is one of continuous substance, and the total pattern is that of an alveolar organization, modified by the presence of the disks.

This interpretation has two advantages: First, it conforms to what is actually observed under the microscope; second, it serves to explain the widely different interpretations given by the other authors noted above. No attempt will be made here to elaborate details as to the nature of the disks, granules, etc., but a few points may be noted. The disks evidently represent regions of high concentration of nucleic acid, and are made of material which is much more resistant to distortion than that of the intervening zones. Presumably the achromatic material is produced within the chromosome during its development. Apparently there are at least two fundamentally different types of chromatic granules. In one type each granule contains material from two different disks (Metz, 1935, p. 491; Metz and Lawrence, 1937) (Fig. 2, C).

Not enough emphasis has been laid on the fact that no criterion is known for distinguishing a really *single* disk or telling how many disks are actually involved in many if not most of what are ordinarily referred to as individual disks.   Many of the latter are known to be compound, but the extent of the complexity is unknown.   Until such fea-
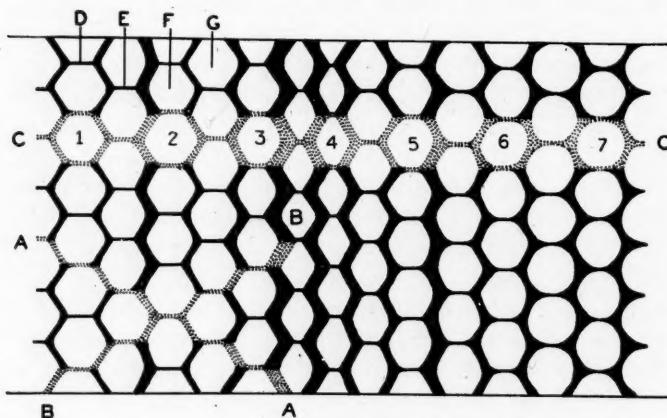

Fig. 1.   Diagram of internal structure of salivary gland chromosome according to the interpretation of Metz and Lawrence.   Compare with the photographs shown in Figs. 3 and 4.   The diagram indicates also how this interpretation serves to explain the conflicting views of other authors.   The transverse (vertical) zigzag bands represent the chromatic disks, separated from one another by achromatic droplets, which, in optical section, may appear spherical, as at the right, or hexagonal, as at the left.   Compare the latter region with region *c* in the photograph in Fig. 3.   The diagonal zigzag lines, such as A–A and B–B, represent what some authors (*e.g.*, Koltzoff, Bridges, Bauer) have considered to be chromonemata.   The series of lines and spaces *C–C* illustrate what Painter and Griffen consider to be a chromonema (bundle).

tures are cleared up the real nature of the "granules," etc., will remain obscure.   A disk may be made up of a multitude of sub-microscopic chromomeres, but according to our evidence no chromomeres of genetic or biological significance are visible.   Rather, the granules, etc., appear to be due to localized distortions of the disks.

Such an interpretation not only conforms to the evidence, but also seems entirely in keeping with expectation when the size and general nature of the giant chromosomes

are considered.   If a chromonema in an "ordinary" dipteran chromosome were extended to the length of a salivary gland chromosome it should be sub-microscopic throughout its length.
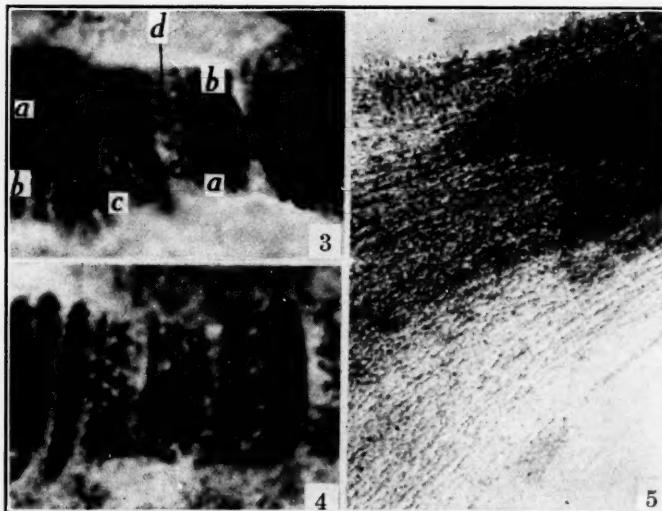
Figs. 3, 4, 5. Photographs of chromosomes and cytoplasm from aceto-carmine smear preparations of salivary glands of *Chironomus* sp.   Figs. 3 and 4, × 2500; Fig. 5, × 1134.   Fig. 3. Portion of one chromosome (pair) showing details of structure.   Note the zigzag lines extending diagonally between *a* and *a* and between *b* and *b*, forming a crisscross pattern (compare with Fig. 1, A–A, B–B).   Also note the relation of these lines to the honey-comb type of pattern shown at *c*, where enough of the achromatic droplets happen to lie in one plane to reveal the pattern clearly (compare with Figs. 1 and 2, B).   At *d* rows of granules are present like those shown schematically in Fig. 2, C.   Fig. 4. Portion of another chromosome (pair) in which the diagonal crisscross lines are accentuated by slight distortion.   The criss-cross pattern is present at all optical levels as one focusses up and down, showing that the lines can not represent chromonemata.   Fig. 5. Stretched cytoplasm, showing how distortion produces granular thread-like striations like those produced by similar means in the chromosomes and thought by some authors to be chromonemata.

In comparing the view of Bauer with that of Painter and Griffen, it is to be noted first that the actual part of the visible pattern which Bauer calls a chromonema is distinctly

different from that which the latter authors call a chromonema (bundle).   This has been determined not only by study of our material, but by examination of preparations kindly provided by Dr. Bauer and Dr. Painter.   The essential aspects of the difference are shown schematically in Fig. 1, where the diagonal lines, such as *A–A* and *B–B*, represent the "chromonemata" of Bauer, and the longitudinal series of lines and spaces, such as illustrated by *C–C*, represent the "chromonemata" (bundles) of Painter and Griffen.

Although the lines of Bauer often do seem thread-like in appearance after certain fixatives, it seems clear from several lines of evidence that they can not be chromonemata, and, indeed, are not actually threads.   *E.g.,* (1) Viewing the chromosome from the side and focussing up and down, these lines are seen to form a diagonal crisscross pattern at all optical levels, indicating that they are a part of a geometrical, three-dimensional system—a meshwork or spongework reflecting an alveolar structure, as indicated in Figs. 1, 3 and 4.   (2) Individual lines extend across both members of the pair of chromosomes, whereas a chromonema is part of a single chromosome.   (3) The number of lines, like that of the granules, differs greatly in different parts of a chromosome and is not what would be expected if they represented chromonemata, as indicated in earlier papers (*l.c.*).   Other reasons are noted below in connection with the interpretation of Painter and Griffen.

Since Painter and Griffen now agree with Metz and Lawrence that no individual chromonemata are detectable in the giant chromosomes the difference in interpretation here is not very significant.   In attempting to throw light on the physical nature of genes and chromonemata it makes relatively little difference whether we consider a disk as made up of a thousand or more individually invisible units (chromomeres) or of a few granules or "chromomeres," each of which is itself made up of a large number of these invisible units.   It should be noted, however, that our evidence does not support the view of Painter and Griffen.

This feature will be treated more fully elsewhere; but a few points may be noted briefly here, as follows: (1) If individual bundles of chromonemata were formed, as postulated by Painter and Griffen, the number should be uniform throughout the chromosome. But, as is well known, the number of longitudinal lines, of chromatic granules and of heavy-walled droplets or so-called "chromomeres" differs widely in different parts of a chromosome. This is true during the development of the chromosome as well as at the end. Also, we have been unable to find evidence that the numbers are regularly multiples of two (8, 16, 32, etc.) as postulated. (2) The evidence of Painter and Griffen is based primarily on severely stretched chromosomes. According to our observations, such evidence is unreliable. In such material the more delicate structure is distorted and gives the appearance of strands, connecting the heavier granules and heavy-walled droplets in the more chromatic regions. The same type of image is produced by stretching the cytoplasm of these same cells (Fig. 5). Moreover, if these delicate lines were really chromonemata they should be thicker and very conspicuous in less stretched conditions, which is not the case. (3) A bundle of chromonemata, on the view of Painter and Griffen, is said to be represented by a continuous thread, on which are aligned granules and conspicuous heavy-walled droplets called "chromomeres." Thus in the pattern of the relaxed chromosome, as shown in Fig. 1, spaces 1 to 7 represent the "chromomeres" of one "chromonema" bundle, and the corresponding spaces at levels 1, 2, 3, etc., represent the "chromomeres" of the other "chromonemata" bundles. Hence, line D and the other lines in this transverse (vertical) row would represent the sides of "chromomeres," while E and its counterparts would represent threads. Similarly, space F, like 2 and its other counterparts, would represent a "chromomere," whereas G, which looks just like it, would merely represent a space between two transverse rows of chromomeres. We are unable to find justification for these distinctions (see Fig. 3). (4) By stretching the chromosome diagonally, side-

wise, etc., the components of the pattern within the chromosome may be lined up into so-called "chromonemata" extending in any desired direction. (5) At some loci conspicuous granules lie *between* what are said to represent chromonemata. Such a condition is shown in Fig. 2, C, which may be interpreted for this purpose by comparison with Fig. 1. *E.g.*, position *D* in Fig. 1, on the view of Painter and Griffen, lies between abutting "chromomeres" of two adjacent chromonemata. This is the position occupied by the granules, such as *d*, in Fig. 2, C. In some cases the droplets, between which such granules lie, are heavy-walled and are clearly the type of structures called "chromomeres" by those authors. (6) According to our evidence from direct observation and also from study of small deficiencies the so-called "chromomeres" are not biological units. It takes two disks to make a transverse plate of "chromomeres," as indicated in Fig. 1.

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SHORTER ARTICLES AND DISCUSSION
THE GENETICS OF NON–EPITHELIAL TUMOR FORMATION IN MICE

THE present communication represents a condensed summary of a series of experiments on the genetics of the tendency to produce non-epithelial tumors in mice.

A total of 343 such tumors occurring spontaneously in two inbred strains of mice and their hybrids is included in the recorded data. Each tumor has been examined and identified histologically.

In one of the parent inbred strains C57 black there were 121 such tumors. In the other dba, dilute brown there were 23.

In the C57 black stock the tumors may be tabulated as in Table 1.

TABLE 1

Type of animal	Total mice	Non-tumor	Non-epithelial tumor	Per cent. showing non-epithelial tumor	Mean age at death non-tumor	Mean age at death tumor	Per cent. of non-epithelial tumor in lymphatic system	Per cent. of non-epithelial tumors in liver
Breeding ♀	570	499	64	11.22	608.1	706.3	57.8	25.0
Virgin ♀	133	109	26	19.54	813.7	711.0	65.4	19.2
Males	174	142	31	17.81	720.2	741.5	67.7	32.3
Total	**877**	**750**	**121**	**13.79**				

The first point to be noted is that there is no significant difference in the incidence of tumors between breeding females, virgin females and males. This is a marked contrast with the behavior of epithelial mammary tumors, which are confined to the female sex and which are much commoner as a general thing in breeding females than in virgins.

The twenty-three tumors which occurred in the other parent stock (dba) were located as follows: lymphatic, 82.6 per cent.; liver, 4.3 per cent.; other regions, 13.1 per cent.

HYBRIDS BETWEEN C57 BLACK AND DILUTE BROWN STOCKS

The only types of hybrids included in this report are virgin females. There are four groups of such animals.

These groups are as follows: F_1 produced from a cross of dilute

brown females by C57 black males (dBF₁) ; F₁ from the recipro-
cal cross of C57 black females by dilute brown males (BdF₁), F₂
hybrids produced by inbreeding dBF₁ mice (dBF₂) ; F₂ animals
derived from matings of BdF₁ animals (BdF₂).

The numbers recorded for each of the four types are given in
Table 2.

TABLE 2

Hybrids	Non-tumor	Tumor	Total
dBF₁	37	5	42
BdF₁	158	43	201
dBF₂............	407	61	468
BdF₂	571	90	661
Total hybrids	**1,173**	**199**	**1,372**

The location of the tumors in the four hybrid generations can
be shown in Table 3.

TABLE 3

	Lymphatic system	Liver	Other sites
dBF₁	40.0	0.0	60.0
BdF₁	37.2	4.7	56.1
dBF₂	44.3	1.6	54.1
BdF₂	36.7	13.3	50.0

The distribution in the hybrids is distinctly different from that
in the two parent strains.   These are given in Table 4.

TABLE 4

	Lymphatic	Liver	Other sites
C57 Blk	62.0	25.6	12.4
Dilute brown	82.6	4.3	13.1

From these figures it is clear that the hybrid animals show a
much more scattered distribution of tumors than do the pure
strains.   In this respect there is no outstanding difference be-
tween the reciprocal crosses.   Both show between 49 and 61 per
cent. of the tumors in situations other than the lymphatics or
liver, while the pure strains showed between 12.3 and 13.2 per
cent.

As regards the *types* of tumors there are also some interesting
data.

The pure dilute brown stock included in its total of 23 tumors
only two types.   The C57 black stock in 121 tumors included nine
types.   The five dBF₁ tumors were of two types, while the dBF₂

generation had 8 types. The BdF_1 generation with forty-three tumors showed eleven types, and the BdF_2 had eleven types.

If one tabulates these data according to the derivation of the maternal line of descent he obtains the following:

Types

Dilute brown	2	C57 black	9
dBF_1	2	BdF_1	11
dBF_2	8	BdF_2	11

The numbers are not sufficiently large to provide conclusive results, but the suggestion is that the types of tissue affected seem to depend somewhat more upon the maternal derivation of the hybrid than upon the paternal. It would be interesting, therefore, to carry on further experiments to see whether in this respect the difference was a real one and whether it was influenced by foster nursing, as Bittner has found the incidence of mammary epithelial tumors in mice to be.

As regards the possible relationship between coat color and the incidence of non-epithelial tumors, there is only an indication of a situation that may contain interesting possibilities. This is the incidence of such tumors in intense and dilute mice. It can be tabulated as in Table 5.

TABLE 5

	Intense			Dilute		
	Non-tumors	Tumors	Per cent. tumor	Non-tumors	Tumors	Per cent. tumor
BdF_2 & dBF_2 ...	732	105	11.4	236	46	16.3
Little 1934 ...	264	13	4.7	115	10	8.0
Total	996	118	10.6	351	56	13.8

If there is a real excess of tumors among dilute mice it is probably due to some physiological relationship rather than to genetic linkage. Its significance is, of course, very doubtful and must await larger numbers for confirmation.

C. C. LITTLE

ROSCOE B. JACKSON MEMORIAL LABORATORY

W. S. MURRAY

STATE INSTITUTE FOR THE STUDY OF
    MALIGNANT DISEASE, BUFFALO, N. Y.

A. M. CLOUDMAN

ROSCOE B. JACKSON MEMORIAL LABORATORY

NOTE OF MODIFICATIONS IN THE MORPHOGENESIS OF *DROSOPHILA MELANOGASTER* OCCURRING UNDER NEUTRON BOMBARDMENT[1]

For several years the authors have been engaged in a fairly extensive qualitative and quantitative investigation of the incidence of white mosaic patches in the compound eyes of imagoes of *Drosophila melanogaster* exposed to x-rays as eggs or young larvae (Enzmann and Haskins, 1935, 1936). The study was undertaken originally as a statistical one, designed to apply the "sensitive volume" technique to a determination of the magnitude of the genic locus involved in the production of white eye color. It soon became apparent, however, that many results of interest could be derived outside of the main line of research from the same types of experiment, notably in connection with the morphogenesis of the adult organs in the larvae of *Drosophila melanogaster*. In consequence, the original research has been extended to include descriptive studies of the qualitative nature of modifications produced by x-rays in developing larvae of the fruit-fly, such modifications becoming evident, for the most part, in the resulting imagoes. Results of such studies, involving a number of strains of fruit-fly, have been and will be published elsewhere (Enzmann and Haskins, 1937).

It became of considerable interest, in this connection, to conduct parallel, and similarly descriptive and qualitative, studies of imagoes of *Drosophila* which had been subjected to neutron bombardment at the same stage of the life cycle. Thanks to the courtesy of Dr. Ernest O. Lawrence it has been possible to carry out such work at the Radiation Laboratory of the University of California, and the generosity of Mr. Everett R. Dempster in collecting eggs, in hatching them and in mailing the developing larvae to us, and of Mr. Paul C. Abersoeld in exposing them to fairly high dosages of neutrons, has permitted the gathering of considerable data. These have shown such marked, and even striking, deviations, qualitatively, from the similar results found with x-rays as to seem worthy of a note at the present time.

The procedure involved in gathering eggs, in segregating retained ova from those freshly fertilized, in rearing the larvae and examining the adults, was essentially that which has been described elsewhere (Enzmann and Haskins, 1935, 1936), in connec-

tion with the x-ray studies. We benefited greatly in this connection from technique in egg collecting devised by Mr. Dempster.

The types of effects observed in the resulting imagoes were quite different in kind, for the most part, from those observed in x-rayed stocks. Reduplication of organs has been found in a very high percentage of modified flies, an effect observed rarely in x-rayed stocks, and suggestive of a modification of possible "organizers" by neutron bombardment. These duplications have been found to involve characteristic bristles, arista, antennae, compound eyes, eye rims, legs, wings, halteres and abdominal tergites, and very probably included certain internal organs, which have not, however, as yet been investigated.

It has been possible to collect a rather completely graded series of duplications of the compound eye and of the eye rim, the incidence of such modifications being surprisingly high. These range from a slight constriction of a single eye occurring along a very definite line and possibly corresponding to the boundary line between the upper and lower lobes of the normal eye, to complete separation, either into two eyes with a single rim or into two completely independent structures. Several cases have been observed in which a complete and perfect accessory eye was formed, mounted on a stalk projecting from above the labrum, the lateral compound eye being normal. Histological study indicated that the accessory, centrally placed organ was connected to the brain by a distinct optic tract, and was presumably functional. It was not a heritable variation, of course, occurring purely in somatic tissue.

The rim of the eye is likewise subject to reduplication, in a fashion apparently relatively independent of that of the eye itself. Usually the partial or complete splitting of the eye is accompanied by separation of the rim. When the eye is fully divided, both halves may be included in the same rim. When the accessory eye has reached a certain and apparently fairly definitely critical size, a separate rim is acquired. The rim, however, may be divided when the eye is not. Two, three or even four eye rims have been found in a single fly, some bearing a few facets, and others entirely empty. Such results are suggestive of the lack of dependence of the development of the eye rim upon that of the eye proper, and its possible origin from a separate anlage, localized in the peripodal membrane of the eye disc.

Antennal duplications are of common occurrence and consider-

able interest.   Usually a complete and normal antenna is found
in its proper location, and an accessory structure occurs, situated
either very close to, or actually within, the compound eye.   The
accessory structure, in such cases, ordinarily occupies a very defi-
nite position, usually in a notch of the compound eye directed
toward the base of the normal antenna and devoid of facets.   The
accessory antenna has a tendency to normal development when
situated outside of the compound eye, but is often represented by
a mass of tumor-like cells, revealing their origin as antennal tissue
by pigmentation and the character of the hair structures, when
occurring within the area of the eye.   This too is suggestive of
"organizer" influence.

Interesting cases of partial duplication of organs have been
found.   Halteres are often found "twinned" and mounted upon
a single basal segment.   Modification of the haltere to wing tissue
has been found.   Reduplication of legs and true wings has like-
wise been observed.   Secondary fusion of duplicated parts some-
times occurs.   No attempt at a theoretical interpretation of the
detailed nature of any of these changes has been attempted at this
stage of the investigation.

It seems indicated that the studies with neutron beams may
constitute an extremely useful tool in morphogenetic investiga-
tions of this character, and it is hoped to carry the work much
further.

<div align="right">

E. V. Enzmann
C. P. Haskins
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THE FACET FREQUENCY DISTRIBUTION IN
BAR–EYED DROSOPHILA

The extensive studies on facet number in the Bar series of
Drosophila involving, as they do, statistical methods in the reduc-
tion of data make it desirable that some knowledge of the form
of the facet frequency distribution be obtained.   With the excep-
tion of a graphic presentation of data on Bar in an early study by
Zeleny (1920) in which a marked positive skewness is disclosed,
no statistical analysis has been given.

The present paper deals with an analysis of the facet frequency distributions in two highly inbred but unrelated Bar-eyed stocks which have been used in a number of experimental studies by the writer.   The data for the facet distributions were taken from the control flies in a series of experiments carried out at 28° C. As far as possible all environmental factors known to effect facet number, *e.g.*, temperature, food, crowding, were carefully controlled.   The residual variability in both stocks may be considered largely as the resultant of unknown factors acting at random on the facet-forming processes, coupled with slight fluctuations in the known environmental factors cited above.

The analysis of the data involved the calculation of the first four moments of the distributions and the derivation from these of the statistical constants with their standard errors given in Table 1.   The analytical constants $\sqrt{\beta_1}$ and β_2 and the criterion,

TABLE 1

ANALYTICAL CONSTANTS FOR FACET FREQUENCY DISTRIBUTIONS

	A males	A females	B males	B females
N	426	354	531	510
Mean ± s.e.	50.81 ± 0.329	48.50 ± 0.357	68.81 ± 0.335	52.45 ± 0.224
σ ± s.e.	6.79 ± 0.233	6.71 ± 0.255	7.72 ± 0.237	5.07 ± 0.159
$\sqrt{\beta_1}$ ± s.e.	0.3624 ± 0.1187	0.3032 ± 0.1302	0.1517 ± 0.1063	0.1845 ± 0.1085
β_2 ± s.e.	2.8760 ± 0.2374	3.0430 ± 0.2604	2.8283 ± 0.2126	2.9010 ± 0.2170
κ_1 ± s.e.	−0.6419 ± 0.4758	0.1897 ± 0.5208	−0.4131 ± 0.4157	−0.3001 ± 0.4339
χ^2	22.8088	26.6785	18.0697	15.7319
Degrees of freedom	15	16	14	13
p	0.0889	0.0455	0.2039	0.2649

κ_1, serve to establish the curve type which may best be used to fit the data.   For the normal distribution $\sqrt{\beta_1} = 0$; $\beta_2 = 3$; and $\kappa_1 = 0$.   The values of $\sqrt{\beta_1}$, which serves as a measure of skewness, are small and positive but deviate significantly from zero in stock A.   In stock B the deviations from zero are statistically insignificant.   One may conclude, therefore, that the facet distribution in stock A shows a slight positive skewness.

β_2, which is a measure of kurtosis or peakedness in the distributions, does not deviate significantly from 3 in either stock; nor does κ_1, which is a function of both β_1 and β_2, deviate significantly from 0.   Despite the slight positive skewness in stock A, it is clear that the normal curve of frequency may be applied to the data as a satisfactory approximation.

The calculated curves and observed points for each of the four sets of data are presented in Fig. 1.   Goodness of fit was tested
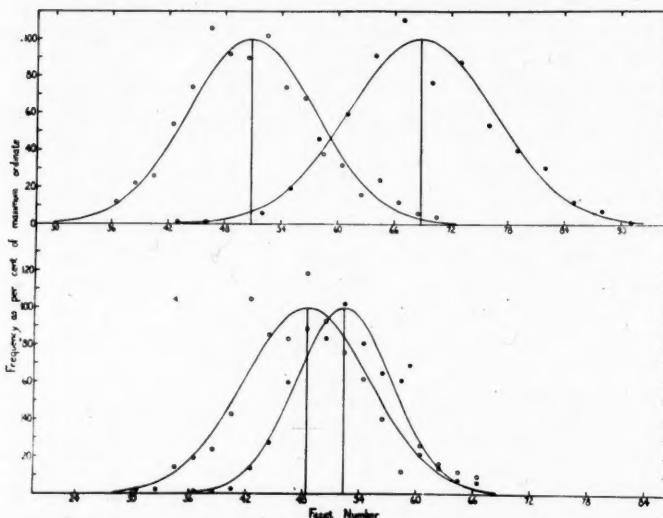

Fig. 1.   Normal frequency curves fitted to facet data of males and females of Stocks A and B.   *Upper Curves*—Males; *Lower Curves*—Females.   *Open Circles*—Stock A; *Shaded Circles*—Stock B.

by the χ^2 method.   The values of χ^2 and the corresponding probabilities are given in Table 1.   The probabilities were taken from Elderton's (1901–02) tables using $(\eta' - 2)$ degrees of freedom.   It is apparent from the probability values that the data for stock B give a much better fit to the normal curves than the data for stock A.   This is to be expected, in view of the slight positive skewness in the data from stock A.   Interpreting the probability values in the conventional manner we may say for stock A males that about 10 of 11 random samples should give as good a fit or better, while for A females about 21 of 22 random samples should give as good a fit or better.   For stock B males 4 out of 5 random samples should give as good a fit or better; for the females about 3 out of 4 random samples should give as good a fit or better.

The better fit of data on stock B to the normal curve may be due to two factors, (1) the greater genetic homogeneity of the stock as shown by a lower parent-offspring correlation (Margolis,

1936) and (2) better control of environmental factors, particularly crowding, which tends to reduce facet number.   Since experiments with stock B were undertaken several years after those with stock A, improvements in experimental technique have probably played an important part.

Zeleny, in the study previously cited, attributed the skewness observed in his distributions to the fact that factors which modify facet number operate exponentially, that is, a ten-facet change in a fly having one hundred facets is equivalent to a twenty-facet change in a fly having two hundred facets.   By using a logarithmic scale in setting up class intervals (each class interval taken as a fixed per cent. of the midvalue of the class) Zeleny found that a very satisfactory approximation to a symmetrical distribution resulted.   This led to the development of a factorial system of notation for facet numbers in which factorial units represented logarithmically equal facet classes.   These factorial units were accepted as a better measure of the factors affecting facet number than facet number itself.

It is clear that the data presented here are symmetrically distributed without recourse to the logarithmic seriation of data recommended by Zeleny.   This difference in the two sets of data requires brief comment.   In the earlier work crowding had not yet been recognized as a factor which affects facet number.   Since this factor causes a reduction in facet number and does not operate at random on the population of developing flies it is precisely the type of environmental factor which will lead to positive skewness.   Those flies affected by crowding are shifted progressively toward the low end of the facet distribution.   From this point of view, recourse to hypothesis concerning the quantitative effect of any factor which modifies facet number is unnecessary.   It is doubtful, moreover, whether a predictable relation can be established between the form of the distribution of a character in a population and the nature of the effect of modifying factors which affect that character, provided that these factors operate at random on members of the population.   The factor of crowding, however, operates selectively on a certain proportion of the population and inasmuch as it causes reduction in facet number leads to positive skewness in the facet distribution.

The analysis presented here demonstrates that the normal frequency curve gives an adequate description of the facet distribution in genetically homogeneous Bar-eyed stocks raised under

satisfactory environmental conditions. Data dealing with the effect of crowding on the form of the facet frequency distribution will be analyzed elsewhere.

OTTO S. MARGOLIS

WESTERN RESERVE UNIVERSITY

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AN EVOLUTIONARY CHANGE IN CHROMOSOME SHAPE IN SCIARA

INTRODUCTION

IF we leave out of account the peculiar "limited" chromosomes of Sciara, which are not involved in the present discussion, there are two common types of chromosome group in this genus. Both have eight chromosomes. One consists of two pairs of V's and two pairs of rods; the other, of one pair of V's and three pairs of rods. Each type is found in several species (Metz, 1926, and unpublished data). Here, as in Drosophila, therefore, it seems evident that rod-like chromosomes become transformed into V's, or V's into rods. The present account deals briefly with the question as to how this transformation is brought about. It is concerned especially with conditions in *S. ocellaris* Comstock. Comparisons of four different "wild" strains have shown that both types of chromosome group are found in this species. The strains are all interfertile. In the present account the results of different crosses are reviewed, together with evidence from study of the salivary gland chromosomes. A description is also given of the chromosome group of the closely related species, *S. reynoldsi* Metz.

Of the various species of Sciara studied to date in this laboratory, *S. ocellaris* and *S. reynoldsi* are the only two which do not possess the so-called "limited" chromosomes (see Metz, 1926, 1938b) and also the only two which are partly interfertile. The cross, *S. reynoldsi* female by *S. ocellaris* male, has never yielded offspring; the reciprocal cross, however, does give an F_1 generation, including males, females and gynandromorphs. (Metz and

Lawrence, 1938, and unpublished data.) Further indication of the close relationship existing between these two species is found in the striking similarity in their external morphology (Metz, 1938a) and also in the fact that the sex-linked character "yellow" in *S. reynoldsi* is allelic to "yellow" in *S. ocellaris* (Crouse and Smith-Stocking, 1938).

The author is indebted to Dr. C. W. Metz for the encouragement and many helpful suggestions he has given throughout the course of this investigation.

MATERIAL

Three different "wild" strains (*i.e.*, laboratory strains descended from wild flies) of *S. reynoldsi* and four different "wild" strains of *S. ocellaris* were available for this study. All the *S. reynoldsi* flies collected thus far have proved to be "bisexual"; that is, single females give both male and female offspring. On the other hand, "unisexual" as well as "bisexual" strains of *S. ocellaris* have been found. Females from the "unisexual" strains give male or female families. "Exceptional" males or females may be found in "unisexual" families. It might be pointed out that certain "bisexual" lines have been developed by selection from "unisexual" strains. The "bisexual" and "unisexual" strains of *S. ocellaris* are interfertile.

The metaphase chromosomes described and figured in this paper are germ-like chromosomes obtained from the gonads of 4- to 6- day old larvae (*i.e.*, 4 to 6 days after hatching of the egg at 74° F). At this stage of development the process of chromosome elimination characteristic of Sciara has occurred; consequently, the germ-like complement in both sexes is composed of four pairs of chromosomes. In the 4- to 6- day old larva the germ cells undergo a series of rapid mitoses. This period of mitotic activity is of somewhat longer duration in the testis than in the ovary.

In making this study, the aceto-carmine smear technique was found to give excellent results. The accompanying illustrations were all made from such preparations.

OBSERVATIONS

(a). *S. reynoldsi*. The metaphase group in the three wild strains of this species consists of two pairs of V-shaped chromosomes and two pairs of rods. One pair of rods is only slightly

longer than the other pair, while the larger pair of V's is nearly twice the size of the small V's. As explained below, it is clear that both pairs of V's are autosomes and that the sex chromosomes are a pair of rods.

(b). *S. ocellaris*—"*bisexual.*" The two "bisexual" strains of *S. ocellaris* have a metaphase group like that of *S. reynoldsi* just described (Fig. 1, *c*). Aceto-carmine smears of brains from

Fig. 1. Metaphase figures from larval gonads. Camera lucida drawings made with 1.4 mm objective and 15 × ocular at table level. Reduced one fourth.

a, b—*S. reynoldsi*, figures from two different wild strains.

c—*S. ocellaris*, figure from "bisexual" strain.

d—*S. ocellaris*, figure from "unisexual" strain.

e, f—*S. ocellaris*, F_1 from the cross of the 2–V strain by the 4–V strain. This 3–V configuration is found also in "unisexual" stock cultures (see text).

male larvae of "bisexual" *S. ocellaris* show seven chromosomes—the missing chromosome being one of the longer rods. Since in the male soma of Sciara only the maternal sex chromosome is normally present, it seems clear that the X is rod-like here and that the V's are all autosomes. This condition differs from that found in *S. pauciseta*, where the X is apparently V-shaped (Schmuck, 1934).

(c). *S. ocellaris*—"*unisexual.*" In "unisexual" strains of *S. ocellaris* two different metaphase configurations are found. There are either three pairs of rods and one pair of large V's (Fig. 1, *d;* Fig. 2, *c*) or two pairs of rods, one pair of large V's and a single small V and rod (Fig. 1, *e, f*).

(d). *S. ocellaris—F₁ from cross of the 2–V strain by the 4–V strain.* Since the "unisexual" and "bisexual" strains of *S. ocellaris* are interfertile, the metaphase configuration in the F_1 larvae from a 2–V female by a 4–V male (reciprocal cross made also) was determined. Here, as expected, three V's and five rods are found, including one pair of large V's, two pairs of rods and a single small V and rod. (See *e* and *f* of Fig. 1 and *d* of Fig. 2.) It is to be noted that the single rod in this metaphase configuration appears to be of the same length as the small V chromosome. At first it was supposed that the small V had originated from the rod, or *vice versa*, through the occurrence of a large inversion.
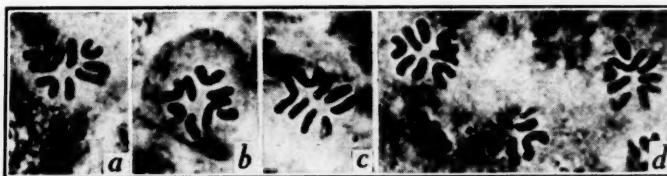

FIG. 2. Photographs of metaphase groups in larval gonads. 1750X.
  a—*S. reynoldsi.*
  b—*S. ocellaris,* figure from "bisexual" strain.
  c—*S. ocellaris,* figure from "unisexual" strain.
  d—*S. ocellaris,* F₁ from the cross of the 2–V strain by the 4–V strain.

Examination of the salivary gland chromosomes of these 3–V larvae, however, revealed no such inversion; instead, essentially normal, synapsed chromosomes were found. Presumably, therefore, the small V arose from the rod, or *vice versa,* through translocation of the spindle fiber locus. The finer cytological details here have not yet been worked out.

DISCUSSION

Certain unpublished data of Mr. C. B. Davidheiser suggest that the genetic determination of "bisexuality" and "unisexuality" in *S. ocellaris* is autosomal rather than sex-linked. Since the heteromorphic chromosomes are autosomes and since all the "bisexual" strains of both *S. ocellaris* and *S. reynoldsi* investigated to date show the same 4–V, 4-rod configuration, while neither of the "unisexual" strains of *S. ocellaris* shows this configuration, it seems possible that the chromosome pair under consideration may be responsible for this determination. The

occurrence and origin of the 3–V, 5-rod configuration found in
"unisexual" stock cultures of *S. ocellaris* have yet to be studied
in detail.

Summary

(1) In *S. ocellaris* two types of chromosome group are found:
four V's and four rods in the "bisexual" strains and two V's
and six rods in the "unisexual" strains.

(2) In the closely related species, *S. reynoldsi*, only the
four-V, four-rod group has been found. All the strains of this
species collected to date are "bisexual."

(3) In both *S. ocellaris* and *S. reynoldsi* the two pairs of V's
are autosomes, while the sex chromosome pair is rod-like.

(4) The "bisexual" and "unisexual" strains of *S. ocellaris*
are interfertile. By crossing the 2–V and 4–V strains of this
species, an F_1 generation showing three V-shaped chromosomes
and five rods can be obtained. The salivary gland chromosomes
of these F_1 larvae give no indication of the origin of the small
V through a large inversion in the rod, or *vice versa*. Pre-
sumably, therefore, the small V arose from the rod, or *vice versa*,
through translocation of the spindle fiber locus.

(5) The genetic significance of the heteromorphic chromo-
some pair in *S. ocellaris* and its relationship to the determination
of "bisexuality" and "unisexuality" in this species has yet to
be investigated.

HELEN V. CROUSE

DEPARTMENT OF EMBRYOLOGY,
   CARNEGIE INSTITUTION OF WASHINGTON,
      BALTIMORE, MD.

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